Cayman CHEMICAL

EPIGENETICS & GENE REGULATION

Tom Brock, Ph.D

Introduction to Epigenetics and Gene Regulation

You can hear it in their former names. Some enzymes were called the "Multiple Myeloma" protein or "Monocytic Leukemia" factor, before they were found to add methyl or acetyl groups to histones. Others were christened "Amplified in Squamous Cell Carcinoma" and "Cancer/Testis Antigen 31", before they were shown to demethylate histones. These are important enzymes from a disease point of view. Perhaps the scientists who were creating these names were trying to balance their excitement, having discovered a key contributor to cancer, with a caution not to overstate their findings. In some cases, their initial analyses, performed in the 1990's, predicted the presence of PHD fingers. At the time, these zinc finger-like domains had been identified in proteins, in *Drosophila*, that were associated with chromatin-mediated transcriptional regulation. PHD fingers were also identified in several mammalian proto-oncogenes, suggesting that it was an oncogenic motif. With each of these first reports, the authors were obliged to report that they did not know the function of the gene product. Still, when they suggest that their discovery may "play an important role in carcinogenesis", the excitement is clear.

It's interesting that in those studies of over a decade ago, the researchers recognized that proteins with motifs for transcriptional regulation "played roles" in cancer, rather than were the direct cause. This phrasing anticipated our current understanding that there are classes of enzymes which 'mark' DNA and protein, epigenetically regulating the expression of clusters of genes. The past few years have seen an explosion of research surrounding the processes that regulate the complexity of the 'histone code' and DNA methylation. Already, we are seeing this expanding world moving beyond chromatin, with methylation and acetylation of RNA and a multitude of proteins, including transcription factors themselves.

This Epigenetics and Gene Regulation mini-catalog highlights the most recent small molecular tools and validated assays developed by Cayman for research. Additional information regarding products listed in this catalog, as well as additional reagents, antibodies, and assays available from Cayman Chemical, may be found at **www.CaymanChem.com**.

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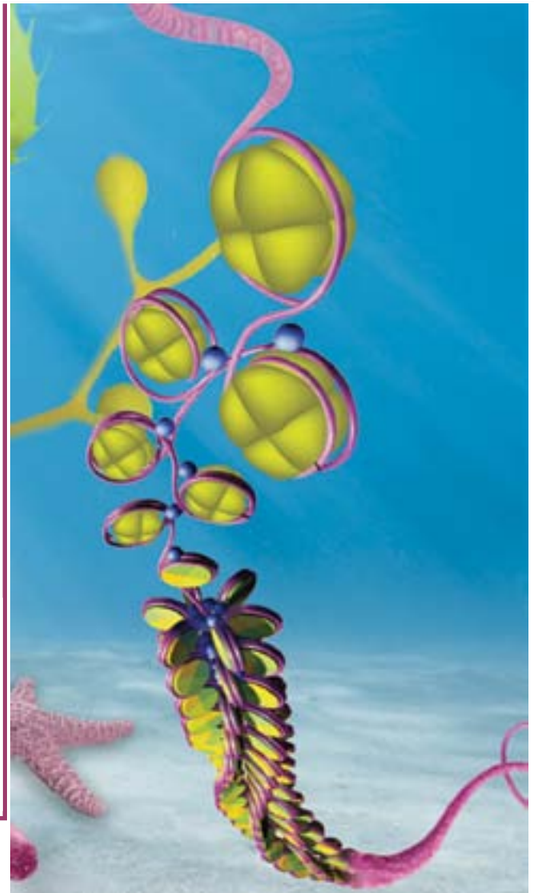
Epigenetics & Gene Regulation



warranty and limitation of remedy

ordering information

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abbreviations

cAMP	Cyclic Adenosine Monophosphate
CFSE	5-(6)-carboxyfluorescein diacetate succinimidyl ester
ChIP	Chromatin Immunoprecipitation
CRE	Cyclic AMP-Responsive Element
CREB	cAMP Response Element Binding
DNA	Deoxyribonucleic Acid
dsDNA	Double Stranded Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
ER	Estrogen Receptor
ERK	Extracellular Signal-Regulated Kinase
FITC	Fluorescein Isothiocynate
FXR	Farnesoid X Receptor
HAT	Histone Acetyltransferase
HDAC	Histone Deactylase
HIF	Hypoxia Inducible Factor
His	Histidine
HIV	Human Immunodeficiency Virus
H_2O_2	Hydrogen Peroxide
ICC	Immunocytochemistry
IF	Immunofluorescence
IHC	Immunohistochemistry
IKK	Inhibitor of Nuclear Factor □B Kinase
IL	Interleukin
IP	Immunoprecipitation
LPS	Lipopolysaccharide
LSD	Lysine-specific Demethylase
LXR	Liver X Receptor
NF-□B	Nuclear Factor-□B
PE	Phycoerythrin
PPAR	Peroxisome Proliferator-activated Receptor
PRMT	Protein Arginine Methyltransferase
miRNA	microRNA
MT	Methyltransferase
RNA	Ribonucleic Acid
SAM	S-adenosyl Methionine
SIRT	Sirtuin
SREBP	Sterol Regulatory Element-Binding Protein
TNF	Tumor Necrosis Factor
WB	Western Blot

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Ep

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Nurturing the Concept of Epigenetics

Being an 'epigenetics researcher' must be like being a spy: you can't tell anyone what you do. It's not that it's dangerous to reveal your identity. It's just that you know you're going to get that blank look, followed by that awkward silence as you try to figure out if it's worth explaining what 'epigenetics' is. It's almost like you should have a false identity, just so you can carry on a conversation. Like a spy, the epigenetics researcher trades the excitement of a thrilling career for a life of secrecy from all but his closest colleagues.

Of course, it doesn't have to be this way. The veil of mystery would start to dissolve if better terminology were used. The term 'genetics' is daunting enough for most people, so 'epigenetics' can only be more bewildering. Perhaps today's scientists could take a lesson from yesteryear's psychologists, who popularized the terms 'nature' and 'nurture' for the genetic and environmental contributions to shaping a person's psyche. In many ways, epigenetics is simply the nurture side to the nature of genetics. This article is intended to introduce some interesting thoughts related to epigenetics and, perhaps, help you understand what interests your neighborhood epigenetics researcher.

The 'Nature' of Genetics

One of the oldest concepts regarding human behavior is the *tabula rasa*, or blank slate. The idea is that, at birth, the human intellect starts from nothing and develops through experience and education. In a sense, intelligence or behavior reflects past inputs from environment. The interplay of one's surroundings, or 'nature', with learning is recognized for its importance worldwide. Moreover, the basic tenets regarding how interactions between nature and the individual shape behavior have been popularized and are part of everyday parlance. How does nature interact with genes?

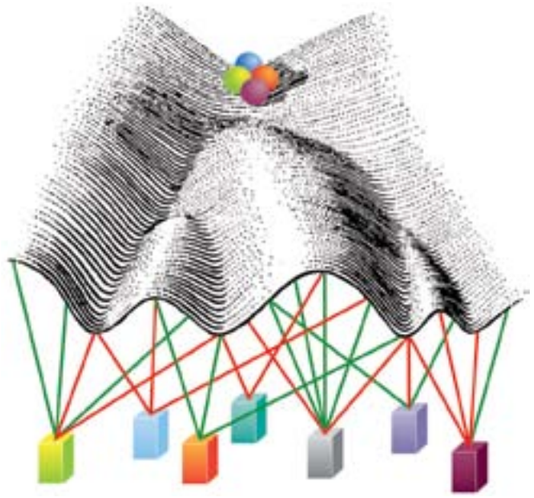

Figure 1. Waddington's model of the epigenetic landscape

Over 200 years ago, Jean-Baptiste Lamarck presented his theory regarding the inheritance of acquired characteristics. There were two central concepts to what has come to be called 'Lamarckian inheritance'. First, changes in physical characteristics due to use or disuse by an individual could be passed on to offspring. Second, some external force drove organismal development up a ladder of complexity. Both ideas look toward the environment affecting the individual. Although the theory never gained general acceptance, it's interesting that it remains standard fare for today's introductory biology classes.

Of course, the field of genetics centers on the actions of genes. Genes provide continuity and a degree of permanence, passing in predictable ways from parents to offspring, from cell to dividing cell. Genes can be detected and sequenced, their frequencies quantified. Much more elusive, though, are the effects of environment on genes. Remarkably, in 1932, at a time when genes were recognized as discrete heritable units but their structure and function unknown, Conrad Hal Waddington used the term 'epigenetics' to refer to the external manifestation of genetic activity. He presented the 'epigenetic landscape' as a way to visualize the forces affecting cell differentiation (Figure 1). In this model, marbles (cells) move varying ways down a landscape whose contour is affected by genes. Details within the contours are further defined by factors above ('epi-') the fixed genetic level, and these details determine the final resting state of differentiation for each cell type.

Whether epigenetic factors act above, below, before, or after the gene depends on the factor. More importantly, 'epigenetics' today commonly refers to changes that are heritable but do not involve changes in the DNA sequence. Specifically, these are changes that affect gene expression, without changing DNA sequence, which can be passed on at least one generation. For single cells, epigenetic changes simply need to be passed through mitotic divisions. For complex organisms, they are changes that are passed on to a later point in life or, better yet, to offspring.

Sharpening the Vision

Like the field of genetics, epigenetics encompasses a diverse array of disciplines. Population epigenetics is concerned with, broadly, the prevalence and importance of epigenetic variation in the natural world. Cellular epigenetics (also called epigenetic mitotic inheritance) would be an appropriate term for the diverse mechanisms, aside from DNA variation, involved in cellular differentiation. Molecular epigenetics would possibly focus on the chemical events that occur on biomolecules that persist through generations. These events, referred to as 'marks', affect gene expression but do not include changes in DNA sequence. The most commonly studied marks include methyl groups on DNA, methyl groups on histones, and acetyl groups on histones. Other enzymatically-mediated modifications of DNA and DNA-associated proteins may also be directly relevant. Importantly, changes in the expression of miRNA, presumably dependent on the above-mentioned marks, can also be central to transgenerational changes. The aggregate of all of these factors constitutes the epigenome.

DNA methylation has long been known as a mechanism for regulating gene expression. The methylation of DNA on cytosine is now viewed as an important mechanism for producing epigenetic changes, because these types of marks can be conserved through mitotic and meiotic cell divisions. While the details behind the regulation of DNA methyl marks are still being studied, a number of points are becoming clear. DNA methylation

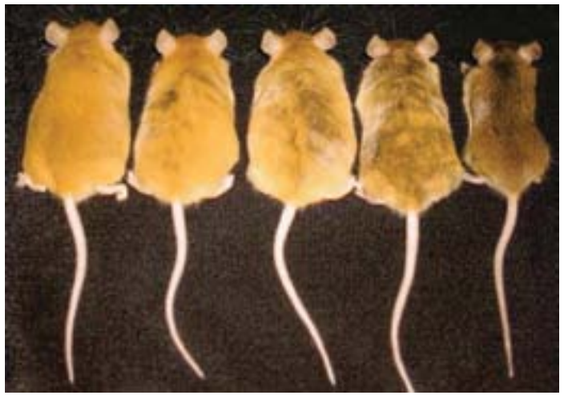

Figure 2. Genetically identical week 15 littermates representing coat colors ranging from agouti (left) to pseudoagouti (right). Note differences in size.*

is recognized to occur on CpG sites, which are cytosine-guanosine pairs on a single DNA strand linked by a phosphate group (as opposed to cytosine-guanosine pairs joining two DNA strands). More recently, cytosine methylation has been shown to occur in either CHG or CHH contexts, where H = A, C, or T. In a recent study examining the DNA methylome (*i.e.,* the complete collection of all DNA methylated sites), Lister, *et al.* reported that some 25% of DNA methylation occurs on CHG or CHH sites in human embryonic stem cells.[1] This non-CpG methylation virtually disappears following differentiation of these stem cells and is restored in induced pluripotent stem cells. The loss of non-CpG methylation and other changes in the DNA methylome were central to changes in phenotype associated with cell differentiation. Thus, DNA methylation is one example of a change which can persist through multiple cell divisions to affect a change in phenotype.

Two Popular Epigenetics Stories

You are what you eat, but does your diet affect your children's children? Bygren, Kaati, and Edvinsson studied a cohort of individuals born in 1905 in rural northern Sweden, where annual harvests are heavily impacted by the weather.[2] County records provided birth and death dates for the 1905 cohort, their parents, and their grandparents. Additional records indicated years of poor, moderate, or superior availability of crop food during the preceding century. Was the lifespan of the individuals born in 1905 affected by the nutritional experience of their predecessors? To focus this question, the authors hypothesized that, in order for famine or food surplus to have persistent effects, the dietary impact must occur in a sexually formative period. Interestingly, they found a significant correlation between food availability for paternal grandfathers when they were 9-12 years old and the survival of their grandchildren. Perhaps more remarkably, grandchild lifespan shortened if there was an excess of food for the paternal grandfather and increased if the grandfather experienced famine during this critical developmental stage. A follow-up study reported that a paternal grandfather experiencing famine during the critical 9-12 years of age passed protection against cardiovascular disease to his grandchildren.[3]

Moreover, those enjoying superior crop years had increased the risk of death due to diabetes for their grandchildren. All correlations were only found down the male line. These studies, less than a decade old, have already become part of epigenetic lore, even though they are purely correlative. However, the results suggest a fun follow-up to the old genetics joke that you should choose parents with good genes: be sure to select a father who starved his dad during his pre-adolescent years.

A series of recent studies have come closer to demonstrating epigenetics in action. While obviously the diet of a pregnant mother can affect fetal development, these studies indicate that dietary supplements, taken by the mother, may mark the genome of the fetus and affect adult health. In the viable yellow agouti (A^{vy}) mouse, expression of the agouti gene leads to a switch from brown to yellow coat color. Expression of the agouti gene is initiated from a cryptic promoter in a retrotransposon inserted in agouti pseudoexon 1A (PS1A).[4] It's known that cytosine methylation on the transposable element prevents agouti gene expression, producing a brown-coated mouse that is referred to as 'pseudoagouti'.[5] Waterland and Jirtle demonstrated that supplementing normal chow given to pregnant mice with methyl donors (folic acid, betaine, vitamin B_{12}, and choline) produced an increase in methylation of CpG sites within PS1A in the offspring and shifted the coat color distribution toward the brown (pseudoagouti) phenotype.[4] These methylation patterns were found in cells from diverse tissues, showing that A^{vy} methylation is determined in the early embryo and maintained with high fidelity throughout development. In another study, genistein, an isoflavonoid naturally found in soy, increased both PS1A methylation and frequency of pseudoagouti expression in offspring when added to the mother's diet.[6] Importantly, ectopic agouti expression is associated with adult-onset obesity, diabetes and cancer.[7] Hypermethylation of PS1A, in mice from genistein-fed mothers, persisted into adulthood, decreasing agouti expression, and, remarkably, protecting mice from obesity (Figure 2).[6] Conversely, hypomethylation of PS1A, following exposure to the common chemical bisphenol A (BPA), increased agouti expression;[8] BPA is known to promote obesity and cancer in mice. Taken together, these results demonstrate that maternal diet has *in utero* effects on the epigenome of the early embryo that can alter susceptibility to disease into adulthood.

These and similar stories are starting to crystallize Waddington's vision of an epigenetic landscape, suggesting that DNA provides the stable base on which our individual details are written in the form of chemical marks. Most likely, the timing, location, and persistency of the marks are some of the variables that will determine how important they will be in impacting such things as development and disease. While much remains to be elucidated, the demonstration that external factors can alter the epigenome suggests that we can manipulate it, hopefully for good rather than evil. In the meantime, continue to pick your parents carefully.

References

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8. Dolinoy, D.C., Huang, D., and Jirtle, R.L. *Proc. Natl. Acad. Sci. USA* **104**, 13056-13067 (2007).

7-AAD Cell Viability Assay Kit 10009856

7-Amino Actinomycin D
Stability: ≥1 year at 4°C
Summary: Cayman's 7-AAD Cell Viability Assay employs 7-AAD as a fluorescent label for dead cells. 7-AAD is a fluorescent dye which is excluded from live cells but penetrates dead or damaged cells to label DNA. Although 7-AAD fluorescence is less intense than that of propidium iodide, it exhibits a higher wavelength emission maximum (excitation at 488 nm, emission at 650 nm) and thus has minimal spectral overlap with PE or FITC. This makes 7-AAD preferable as a viability marker when FITC and/or PE are used simultaneously to label surface or intracellular antigens. A fixative/actinomycin D solution is included in the kit for cell fixation and blocking, making subsequent immunostaining of surface/intracellular antigens possible. This kit provides a convenient tool to quantify cytotoxic effects of environmental toxins or drug candidates.

1 ea

Acetyl Lysine Monoclonal Antibody (Clone 7F8) 10010567

Supplied as: Purified IgG_1 **Stability:** ≥1 year at -20°C
Summary: Antigen: acetylated KLH • Host: mouse • Isotype: IgG_1 • Cross Reactivity: human, murine, rat, bovine, and avian acetyl lysine; pan specific • Application(s): ELISA, ICC, and WB

1 ea

NEW Acetyl Lysine Polyclonal Antibody-biotin 13725

Supplied as: Immunoglobulin in PBS **Stability:** ≥1 year at -20°C
Summary: Antigen: acetylated KLH • Host: rabbit • Cross Reactivity: (+) acetylated lysine residues; (-) non-acetylated proteins • Application(s): ELISA, IF, IP, and WB

400 µl

NEW Acetyl Lysine Polyclonal Antibody HRP Conjugate 13726

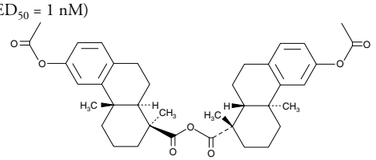
Supplied as: Immunoglobulin in PBS **Stability:** ≥1 year at -20°C
Summary: Antigen: acetylated KLH • Host: rabbit • Cross Reactivity: (+) multi-species • Application(s): ELISA, IF, IP, and WB

400 µl

Acetyl Podocarpic Acid Anhydride 10007686

[344327-48-6] APD
MF: $C_{38}H_{46}O_7$ **FW:** 614.8 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent, semi-synthetic LXR agonist derived from extracts of the mayapple; induces the expression of the ABCA1 reverse cholesterol transporter to increase the efflux of cholesterol from enterocytes and thus inhibits the overall absorption of cholesterol (ED_{50} = 1 nM)

1 mg
5 mg
10 mg
50 mg

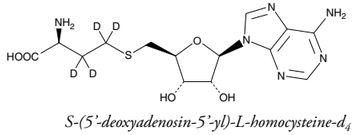


6-(acetyloxy)-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-1-phenanthrenecarboxylic acid, anhydride

NEW S-Adenosyl-L-Homocysteine-d_4 9000372

SAH-d_4
MF: $C_{14}H_{16}D_4N_6O_5S$ **FW:** 388.4 **Chemical Purity:** ≥98%
Deuterium Incorporation: ≤1% d_0
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: An internal standard for the quantification of SAH by GC- or LC-MS

500 µg
1 mg
5 mg

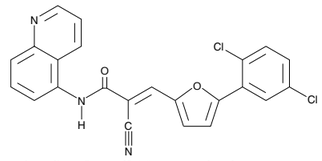


S-(5'-deoxyadenosin-5'-yl)-L-homocysteine-d_4

AGK2 13145

[304896-28-4]
MF: $C_{23}H_{13}Cl_2N_3O_2$ **FW:** 434.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A cell-permeable, selective inhibitor of SIRT2 (IC_{50} = 3.5 µM) that minimally affects either SIRT1 or SIRT3; rescues dopamine neurons from α-synuclein toxicity in both *in vitro* and *in vivo* Parkinson's disease models

1 mg
5 mg
10 mg
25 mg



2-cyano-3-[5-(2,5-dichlorophenyl)-2-furanyl]-N-5-quinolinyl-2-propenamide

NEW Anacardic Acid 13144

[16611-84-0] 6-pentadecyl Salicylic Acid
MF: $C_{22}H_{36}O_3$ **FW:** 348.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An alkyl salicylic acid isolated from cashew shells; inhibits the HAT activity of p300 and p300/CREB-binding protein-associated factor (pCAF) (IC_{50} = 8.5 and 5 µM, respectively); suppresses NF-κB activation, inhibits IκBα phosphorylation, and prohibits p65 nuclear translocation

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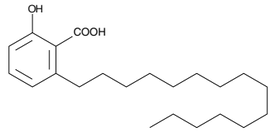
10 mg
25 mg
50 mg



2-hydroxy-6-pentadecyl-benzoic acid

Androgen Receptor (Phospho-Ser[210,213]) Monoclonal Antibody (Clone 156C135.2) 13492

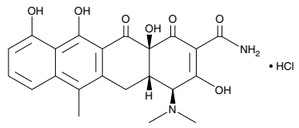
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human androgen receptor amino acids 207-221 • Host: mouse, clone 156C135.2 • Cross Reactivity: (+) human and monkey androgen receptor • Application(s): IHC (paraffin-embedded sections) and WB

1 ea

Anhydrotetracycline (hydrochloride) 10009542

[13803-65-1]
MF: $C_{22}H_{22}N_2O_7$ • HCl **FW:** 462.9 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A powerful effector in both the TetR and revTetR transcriptional regulator systems, binding the TetR 35-fold more strongly than Tet; does not act as a general inhibitor of translation and is a poor antibiotic

10 mg
25 mg
50 mg
100 mg



4-(dimethylamino)-1,4S,4aS,5,12,12aS-hexahydro-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-monohydrochloride-2-naphthacenecarboxamide

ATF2 (Phospho-Ser[490,498]) Polyclonal Antibody 10009410

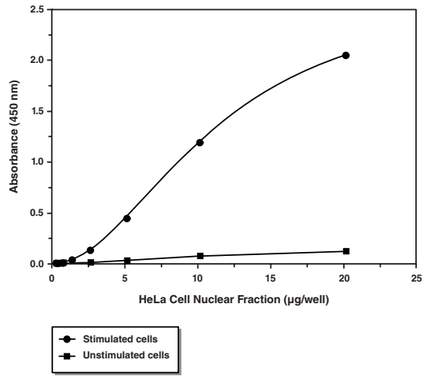
Activating Transcription Factor 2
Supplied as: Peptide affinity-purified antibody **Stability:** ≥1 year at -20°C
Summary: Antigen: phosphopeptide corresponding to amino acid residues surrounding phospho-Ser[490,498] of human ATF2 • Host: rabbit • Cross Reactivity: (+) human ATF2; expected to react with rat ATF2 • Application(s): IHC (frozen sections) and WB

100 µl

ATF2 (Phospho-Thr[69,71]) Transcription Factor Assay Kit 600130

Activating Transcription Factor 2
Stability: ≥6 months at -80°C
Summary: ATF2 is a sequence-specific DNA-binding protein belonging to the bZIP family of transcription factors that bind with high affinity to the octameric CRE. ATF2 mediates both transcription and DNA damage control through its phosphorylation/activation in response to inflammatory cytokines, UV irradiation, alkylating compounds, and other cellular stressors. Cayman's ATF2 (Phospho-Thr[69,71]) Transcription Factor Assay is a sensitive colorimetric method for detecting specific transcription factor binding activity from nuclear exctracts and whole cell lysates. A specific dsDNA sequence containing the CRE is immobilized onto the wells of a 96-well plate. ATF2 contained in a nuclear extract, binds specifically to the CRE. The activated ATF2 transcription factor complex is detected by addition of a specific primary antibody directed against the phospho-Thr[69,71] epitope on ATF2. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

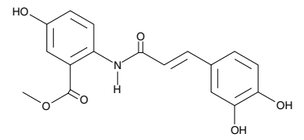
96 wells



Avenanthramide-C methyl ester 10011336

[955382-52-2]
MF: $C_{17}H_{15}NO_6$ **FW:** 329.3 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: Inhibitor of NF-κB activation that blocks the phosphorylation of IKK and IκB (IC_{50} ~40 µM); dose dependently inhibits the expression and secretion of IL-6, IL-8, and MCP-1 in human aortic endothelial cells

500 µg
1 mg
5 mg
10 mg

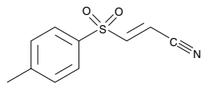


2-[[(2E)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propen-1-yl]amino]5-hydroxy-benzoic acid, methyl ester

BAY-11-7082 10010266

[19542-67-7]
MF: $C_{10}H_9NO_2S$ **FW:** 207.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A selective and irreversible inhibitor of NF-κB activation that blocks TNF-α-induced phosphorylation of IκB-α without affecting constitutive IκB-α phosphorylation; inhibits the TNF-α-induced surface expression of adhesion molecules ICAM-1, VCAM-1, and E-selectin in human endothelial cells (IC_{50} = 5-10 µM)

5 mg
10 mg
25 mg
50 mg

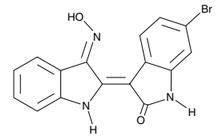


3-[(4-methylphenyl)sulfonyl]-(2E)-propenenitrile

BIO 13123

[667463-62-9] GSK 3 IX, MLS 2052
MF: $C_{16}H_{10}BrN_3O_2$ **FW:** 356.2 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A cell-permeable *bis*-indolo (indirubin) compound that acts as a highly potent, selective, reversible, and ATP-competitive inhibitor of GSK3α/β (IC_{50} = 5 nM); inhibition of GSK activates the Wnt signaling pathway and sustains pluripotency in human and murine embryonic stem cells (ESCs); maintains self-renewal in human and murine ESCs as well as induces the differentiation of neonatal cardiomyocytes

1 mg
5 mg
10 mg
25 mg

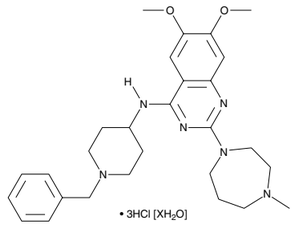


6-bromo-3-[(3E)-1,3-dihydro-3-(hydroxyimino)-2H-indol-2-ylidene]-1,3-dihydro-(3Z)-2H-indol-2-one

NEW BIX01294 (hydrochloride hydrate) 13124

MF: $C_{28}H_{38}N_6O_2$ • 3HCl [XH_2O] **FW:** 600.0 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A selective inhibitor of G9a histone MT (IC_{50} = 1.7 µM); less effectively inhibits G9a-like protein (IC_{50} = 38 µM) and has no effect on other known histone MTs

500 µg
1 mg
5 mg
10 mg



2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-6,7-dimethoxy-N-[1-(phenylmethyl)-4-piperidinyl]-4-quinazolinamine, trihydrochloride, hydrate

Bnc 1 Polyclonal Antibody 13502

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human Bnc 1 within the region of amino acids 330-380 • Host: rabbit • Cross Reactivity: (+) chimpanzee, human, and monkey Bnc 1 • Application(s): WB • Bnc1 is a zinc-finger trascription factor specific for squamous cell epithelium and for the constituent keratinocytes at a stage either prior to or at the very beginning of terminal differentiation.

1 ea

Brd4/HUNK1 Polyclonal Antibody 13497

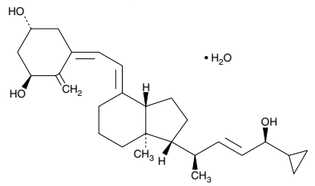
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human Brd4 within the region of amino acids 150-200 • Host: rabbit • Cross Reactivity: (+) chimpanzee, human, murine, and rat Brd4 • Application(s): WB • Brd4 is a chromatin-binding protein whose expression is induced in response to growth stimuli.

1 ea

Calcipotriol (hydrate) 10009599

[112828-00-9] Calcipotriene (hydrate)
MF: $C_{27}H_{40}O_3 \cdot H_2O$ **FW:** 412.6 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A low-calcemic vitamin D receptor agonist; increases keratinocyte differentiation and reduces expression of transcriptional activators in the inflammatory response; induces T-cell differentiation in keratinocytes

10 mg
50 mg
100 mg
500 mg

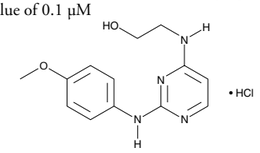


24-cyclopropyl-(1α,3β,5Z,7E,22E,24S)-9,10-secochola-5,7,10(19),22-tetraene-1,3,24-triol, monohydrate

Cardiogenol C (hydrochloride) 13187

[671225-39-1]
MF: $C_{13}H_{16}N_4O_2 \cdot HCl$ **FW:** 296.8 **Purity:** ≥97%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: Induces the differentiation of MHC-positive cardiomyocytes from embryonic stem cells with an EC_{50} value of 0.1 µM

1 mg
5 mg
10 mg
50 mg

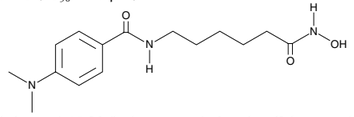


2-[[2-[(4-methoxyphenyl)amino]-4-pyrimidinyl]amino]-ethanol

CAY10398 89740

MF: $C_{15}H_{23}N_3O_3$ **FW:** 293.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of HDAC (IC_{50} = 10 µM)

1 mg
5 mg
10 mg
25 mg

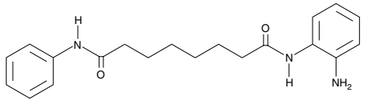


4-(dimethylamino)-N-[6-(hydroxyamino)-6-oxohexyl]-benzamide

CAY10433 10005019

[537034-17-6] BML-210, N-phenyl-N'-(2-Aminophenyl)hexamethylenediamide
MF: $C_{20}H_{25}N_3O_2$ **FW:** 339.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of HDAC with an IC_{50} value of 30 µM when tested in HeLa cell nuclear extracts using 200 µM acetylated fluorometric substrate

1 mg
5 mg
10 mg
25 mg

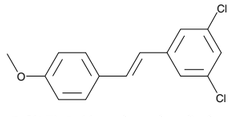


N-(2-aminophenyl)-N'-phenyl-octanediamide

CAY10464 10006545

[688348-37-0]
MF: $C_{15}H_{12}Cl_2O$ **FW:** 278.0 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent and selective AhR antagonist with a K_i value of 1.4 nM

10 mg
25 mg
50 mg
100 mg

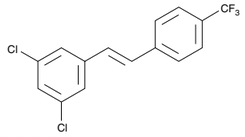


1,3-dichloro-5-[(1E)-2-(4-methoxyphenyl)ethenyl]-benzene

CAY10465 10006546

[688348-33-6]
MF: $C_{15}H_9Cl_2F_3$ **FW:** 317.1 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An analog of resveratrol that acts as a potent and selective AhR agonist (K_i = 0.2 nM)

1 mg
5 mg
10 mg
50 mg

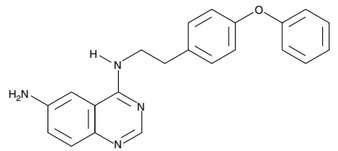


1,3-dichloro-5-[(1E)-2-[4-(trifluoromethyl)phenyl]ethenyl]-benzene

CAY10470 10006734

[545380-34-5]
MF: $C_{22}H_{20}N_4O$ **FW:** 356.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of NF-κB activation with an IC_{50} value of 11 nM in human Jurkat cells; inhibits TNF-α production from LPS-stimulated murine splenocytes (IC_{50} = 7 nM)

500 µg
1 mg
5 mg
10 mg



N^4-[2-(4-phenoxyphenyl)ethyl]-4,6-quinazolinediamine

CAY10512 10009536

[139141-12-1]
MF: $C_{15}H_{13}FO$ **FW:** 228.3 **Purity:** ≥97%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A substituted *trans*-stilbene analog of resveratrol that is 100-fold more potent as measured by antioxidant activity; inhibits TNF-α-induced activation of NF-κB (IC_{50} = 0.15 µM)

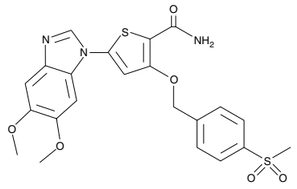
10 mg
50 mg
100 mg
500 mg

1-fluoro-2-[2-(4-methoxyphenyl)ethenyl]-benzene

CAY10575 10011248

[916985-21-2]
MF: $C_{22}H_{21}N_3O_6S_2$ **FW:** 487.6 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A benzimidazole analog that inhibits IKK-α with an IC_{50} value of ~15.8 µM

1 mg
5 mg
10 mg
25 mg

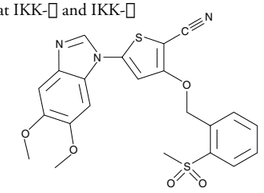


5-(5,6-dimethoxy-1H-benzimidazol-1-yl)-3-[[4-(methylsulfonyl)phenyl]methoxy]-2-thiophenecarboxamide

CAY10576 10011249

[862812-98-4]
MF: $C_{22}H_{19}N_3O_5S_2$ **FW:** 469.5 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A benzimidazole analog that selectively inhibits IKK-β with an IC_{50} value of 40 nM and is essentially inactive at IKK-α and IKK-γ

1 mg
5 mg
10 mg
50 mg

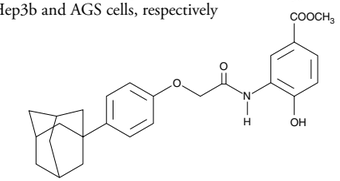


5-(5,6-dimethoxy-1H-benzimidazol-1-yl)-3-[[2-(methylsulfonyl)phenyl]methoxy]-2-thiophenecarbonitrile

CAY10585 10012682

[934593-90-5] Hypoxia Inducible Factor-1α Inhibitor
MF: $C_{26}H_{29}NO_5$ **FW:** 435.5 **Purity:** ≥97%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A novel small molecule inhibitor of HIF-1α accumulation and gene transcriptional activity; inhibits HIF-1 transcriptional activity with IC_{50} values of 2.6 and 0.7 µM in human Hep3b and AGS cells, respectively

1 mg
5 mg
10 mg
25 mg

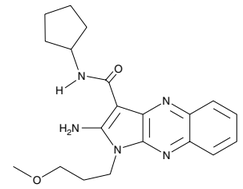


4-hydroxy-3-[[2-(4-tricyclo[3.3.1.13,7]dec-1-ylphenoxy)acetyl]amino]-benzoic acid, methyl ester

CAY10591 10009797

[839699-72-8] SIRT1 Activator 3
MF: $C_{20}H_{25}N_5O_2$ **FW:** 367.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An activator of SIRT1 that decreases TNF-α levels from 325 pg/ml (control) to 104 and 53 pg/ml at 20 and 60 µM, respectively; exhibits a significant dose-dependent effect on fat mobilization in differentiated adipocytes

1 mg
5 mg
10 mg
25 mg

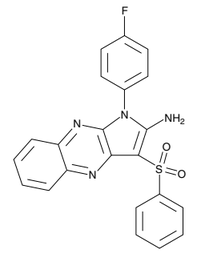


2-amino-N-cyclopentyl-1-(3-methoxypropyl)-1H-pyrrolo[2,3-b]quinoxaline-3-carboxamide

CAY10602 10009796

[374922-43-7]
MF: $C_{22}H_{15}FN_4O_2S$ **FW:** 418.4 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: Derived from high throughput screening for compounds that increase the SIRT1-mediated deacetylation of a SIRT1-specific substrate; dose-dependently suppresses the NF-κB-dependent induction of TNF-α by LPS in THP-1 cells, with approximately 75% inhibition achieved at 60 µM, without cytotoxicity

5 mg
10 mg
50 mg
100 mg

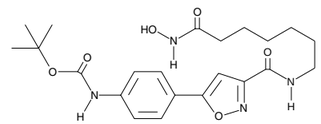


1-(4-fluorophenyl)-3-(phenylsulfonyl)-1H-pyrrolo[2,3-b]quinoxalin-2-amine

CAY10603 13146

[1045792-66-2]
MF: $C_{22}H_{30}N_4O_6$ **FW:** 446.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent and selective inhibitor of HDAC6 (IC_{50} = 0.002 nM, as compared with 271, 252, 0.42, 6851, and 90.7 nM for HDAC1, 2, 3, 8, and 10, respectively); prevents the growth of several pancreatic cancer cell lines (IC_{50} = 0.1-1 µM)

500 µg
1 mg
5 mg
10 mg

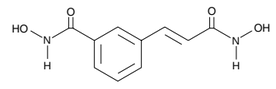


N-[4-[3-[[[7-(hydroxyamino)-7-oxoheptyl]amino]carbonyl]-5-isoxazolyl]phenyl]-1,1-dimethylethyl ester, carbamic acid

CBHA 13172

[174664-65-4] HDAC Inhibitor II, m-Carboxycinnamic Acid bis-Hydroxamine
MF: $C_{10}H_{10}N_2O_4$ **FW:** 222.2 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: HDAC1 and HDAC3 inhibitor (ID_{50} = 0.01 and 0.07 µM, respectively, *in vitro*); induces apoptosis in nine different neuroblastoma cell lines in culture (0.5-4.0 µM) and completely suppresses neuroblastoma tumor growth in SCID mice at 200 mg/kg

5 mg
10 mg
25 mg
50 mg



N-hydroxy-3-[3-(hydroxyamino)-3-oxo-1-propen-1-yl]-benzamide

vol. 8 Ep

Tom Brock, Ph.D.

Histone Methylation:
SET *versus* Jumonji

The battle for control of the genome is one of the longest running and most epic on earth. Perhaps, at one time, it was a simple contest, deciding whether a gene was turned on or turned off. In that primordial organism, there may have been a silencing mechanism to balance the action of an activating enzyme. However, as systems (and the genes that program them) grew more complex, it became necessary to diversify the collections of initiators and terminators, so that groups of genes could be controlled in synchrony. Additional players found roles in fine-tuning gene expression, modulating the magnitude and duration of activation as well as the rate of cessation. One front in this battlefield of action centers on histone methylation. The addition or removal of methyl 'marks' from histones activates or represses gene activity. This article introduces the histones and profiles key players from the SET team of methyltransferases and the Jumonji team of demethylases.

Meet the Histones

A focal point for gene regulation and transcription in eukaryotes is the nucleosome, which consists of some 146 base pairs of DNA wrapped twice around an octamer composed of two sets of the core histones, H2A, H2B, H3, and H4. Remarkably, the human genome contains over seventy genes, clustered primarily on chromosomes 1 and 6, encoding these histones. The proteins themselves are small (~103-136 aa). The C-termini are highly conserved, forming three α-helices separated by loop regions (Figure 1A). The N-terminal tails distinguish the different histones (Figure 1B). The 14-20 different genes for each histone encode sequences that are identical except for minor, conservative substitutions. For example, all genes encoding H2A are identical except for a Ser-Thr variation at residue 16. Histone 1 includes some eleven distinct subtypes and serves as a linker histone, interacting with DNA at the exit or entry end of the nucleosomal core DNA.[1]

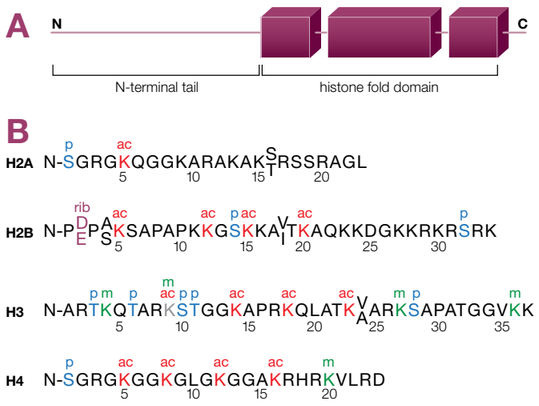


Figure 1. Histone tails
A. Positioning of the histone tail relative to the C-terminal folded region.
B. Amino acid sequences of core histone N-terminal tails, indicating sites of phosphorylation (p), acetylation (ac), ADP ribosylation (rib), and methylation (m).

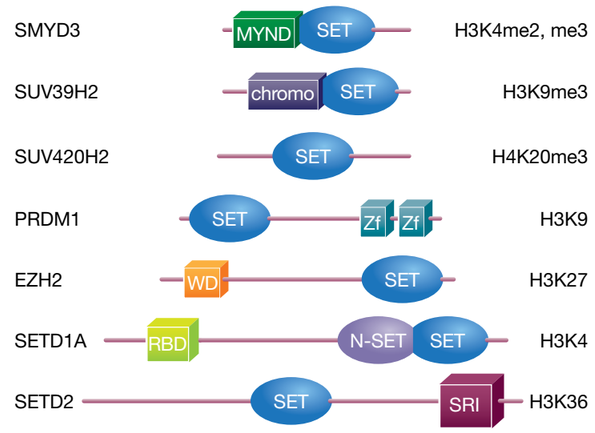


Figure 2. Domain architecture of human SET domain-containing proteins

In the nucleosome, the folded C-termini of the histones interact with one another and the N-termini tails protrude away from this complex. This makes them accessible for post-translational modification, including acetylation, methylation, phosphorylation, and ADP ribosylation. Also, the tails are rich in the basic residues lysine (K) and arginine (R). These positive residues are available to interact electrostatically with the negatively charged phosphate backbone of DNA. Interestingly, they are also positioned regularly, with 2 to 3 uncharged residues typically intervening. Notably, lysine methylation is limited to six sites on H3 and H4. These sites are named by the histone and residue number: H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20. In addition, each site can be mono-, di-, or tri-methylated, adding to the diversity of post-translational modification of histones.

SET Domain Proteins

Many histone lysine methyltransferases (KMTs) contain a SET domain, named after regions shared by three *Drosophila* proteins recognized as being involved in epigenetic processes: Su(var)3-9, Enhancer of zeste, and Trithorax. The SET domain, which is thought to be involved in protein-protein interactions, includes conserved N- and C- terminal regions (SET-N and SET-C) and an intervening insert region (SET-I). Flanking pre- and post-SET regions are typically also required for full KMT activity.

There are dozens of human proteins which demonstrate KMT activity. Many are segregated structurally. For example, the SMYD proteins are short KMTs that contain SET and MYND-type zinc finger domains (Figure 2). Like other zinc finger domains, MYND domains are involved in protein-protein interactions, commonly binding a co-repressor protein, like N-CoR or SMRT. SMYD1 acts as a transcriptional repressor, is essential for cardiomyocyte differentiation and interacts with HDACs. SMYD3 specifically methylates H3K4, inducing di- and tri-methylation, but not mono-methylation.

The human SUV proteins are homologs of the *Drosophila* Su(var) proteins. There are two homologs, SUV39H1 and SUV39H2, that specifically trimethylate H3K9 after it has already been monomethylated. Both proteins contain N-terminal chromatin organization modifier (chromo) domains, which facilitate the condensation of heterochromatin. They function mainly in these condensed heterochromatin regions, suppressing gene expression. Trimethylation on H3K9 facilitates DNA methylation in this context. Two additional Su(var) homologs, SUV420H1 and SUV420H2, specifically trimethylate H4K20. Like the SUV39 homologs, these proteins are targeted to heterochromatin and are involved in epigenetic transcriptional repression.

Another structurally-defined family, the PRDM series, contains a PR domain, an evolutionarily conserved region of about 100 amino acids that is involved in protein-protein interactions. PRDM proteins also contain classical C2H2-type zinc finger domains which mediate DNA binding. PRDM1, also known as BLIMP1, acts as a transcriptional repressor, binding to the promoter of β-interferon, and in this way regulates B cell maturation. PRDM2, also known as RIZ, is another important family member. It methylates H3K9, binds the retinoblastoma protein, and is highly expressed in brain tumors.

The Enhancer of zeste homologs, EZH1 and EZH2, are polycomb group (PcG) proteins that can mono-, di- and trimethylate H3K27. The EZH proteins contain WD repeat binding domains, which mediate interaction with EED (embryonic ectoderm development) protein to form, with SUZ12 (suppressor of zeste 12 homolog), the polycomb repressor complex 2 (PRC2). Both EZH complexes play important roles in embryonic stem cell function.

A diverse group of SET domain-containing proteins is denoted as SETD. Two key members, SETD1A and SETD1B, methylate H3K4, but not if H3K9 is already methylated. Both proteins, which function as components of multimeric complexes, contain RNA binding domains (RBD). SETD2, unlike the SETD1 proteins, methylates H3K36, binds DNA at promoters, and directly binds hyperphosphorylated RNA polymerase II large subunit. This latter interaction is mediated by a Set2Rpb1 interacting (SRI) domain and serves to couple H3K36 methylation with transcript elongation.

Most of the proteins with SET domains tend to methylate a specific amino acid, so they may also be grouped based on target. Some targets with their KMTs (using gene names) include H3K4: MLL1-5, SETD1A-B, SETD7, SMYD1-4; H3K9: EHMT1-2, PRDM2, SETDB1-2, SUV39H1-2; H3K27: EZH1-2; H3K36: SETD2; and H4K20: SETD8, SUV420H1-2. The nuclear receptor-binding SET domain (NSD) proteins act on multiple sites (*e.g.*, NSD1 at H3K36 and H4K20, NSD3 at H3K4 and H3K27).

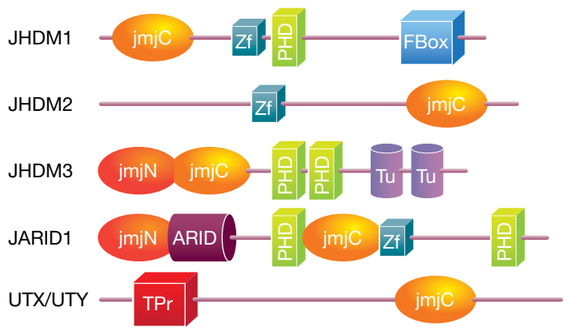


Figure 3. Domain architecture of human JmjC domain-containing proteins

Jumonji's Game

The lysine demethylase (KDM) proteins have multiple names but relatively focused functions. The first KDM to be discovered was called 'lysine-specific demethylase', or LSD1 (*a.k.a.*, KDM1A), and acts on H3K4me2/1 and H3K9me2/1 (di- or mono-methylated K4 and K9 on H3). The majority of other KDMs, which can act on mono-, di-, or tri-methylated substrates, contain the characteristic Jumonji C (JmjC) domain.[2] These can be divided into three groups: the JmjC domain-containing histone demethylation (JHDM) proteins, Jumonji/ARID domain-containing (JARID) proteins, and the ubiquitously transcribed on chromosome X/Y (UTX/UTY) proteins. The JARID proteins target K4, and the UTX proteins prefer K27, on H3. The JHDM proteins can demethylate either K9 or K36 on H3.

A quick search of protein families (search: Pfam), compiled by the Sanger Institute, indicates that the JmjC domain occurs in 1,135 sequences from 131 species, including eukaryotes and prokaryotes. At the German SMART site, the JmjC domain occurs in 1,750 listed proteins, with 20% from bacteria, which is interesting because bacteria don't use histones to organize their DNA. In most KDMs, the JmjC domain binds the key co-factors, iron Fe(II) and α-ketoglutarate. Not surprisingly, this domain is typically found to be essential for demethylase activity. In addition, some KDMs contain an additional N-terminal Jmj (JmjN) domain, which also contributes to enzymatic activity.

The different classes of KDMs contain characteristic patterns of additional domains that facilitate their specific functions (Figure 3). For example, many have zinc fingers (Zf), including the PHD finger. Zinc fingers in general are important in mediating interactions with other molecules, including proteins, DNA, RNA, and lipids. Similarly, F-Box domains and tetratricopeptide repeats (TPr) facilitate intermolecular interactions. Interestingly, paired Tudor (Tu) domains specifically bind methylated histone tails. The abundance of such binding domains indicates that KDMs commonly participate in larger structural and functional complexes.

Differences in the types and arrangements of these interacting domains underlie differences in actions of KDMs.[3] For example, human JHDM1A, which contains a second F-Box, is heterochromatin-associated and represses the transcription of small non-coding RNAs, silencing centromeric satellite repeats.[4] Low levels of this enzyme are found in prostate carcinomas. Human JHDM1B, on the other hand, is a nucleolar protein that represses transcription of ribosomal RNA genes.[5] Low JHDM1B expression occurs in aggressive brain tumors. Compare these JHDM1 proteins with the human JARID1C enzyme, which interacts with some eighteen distinct proteins, including several HDACs and transcriptional repressors REST and NCOR1.[6] Defects in this protein, also known as SMCX, result in X-linked mental retardation in humans.

Of course, the really pressing question must be "What is a jumonji?"! Japanese researchers, led by Toru Higashinakagawa, described a gene mutation which altered neural tube development in fetal mice.[7] The surface of the developing neural plate, which looks like a groove in normal mice, was shaped like a cross in the mutant. The gene was named 'jumonji' which means 'cruciform', derived from the word 'ju', which is the number ten and symbolized by a cross in Japanese. This protein is now known as JARID2.

References
1. Happel, N. and Doenecke, D. *Gene* **431**, 1-12 (2009).
2. Klose, R.J., Kallin, E.M., and Zhang, Y. *Nat. Rev. Genet.* **7**, 715-727 (2006).
3. Lan, F., Nottke, A.C., and Shi, Y. *Curr. Opin. Cell Biol.* **20**, 316-325 (2008).
4. Frescas, D., Guardavaccaro, D., Kato, H., *et al. Cell Cycle* **7**, 3539-3547 (2008).
5. Frescas, D., Guardavaccaro, D., Bassermann, F., *et al. Nature* **450**, 309-313 (2007).
6. Tahiliani, M., Mei, P., Fang, R., *et al. Nature* **447**, 601-605 (2007).
7. Takeuchi, T., Yamazaki, Y., Katoh-Fukui, Y., *et al. Genes Dev.* **15**, 1211-1222 (1995).

Cell Cycle Phase Determination Kit 10009349

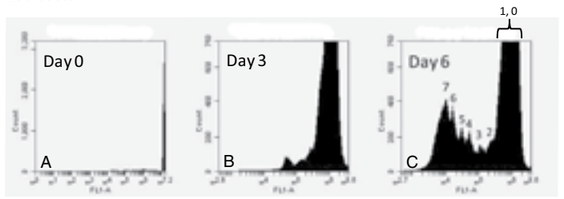
Stability: ≥6 months at -20°C
Summary: Cayman's Cell Cycle Phase Determination provides an easy to use tool for studying the induction and inhibition of cell cycle progression in any cell suspension sample. The assay involves the fixation and permeabilization of the cells of interest, making possible the staining of DNA within intact cells by propidium iodide. This kit will allow the investigator to determine the percentage of cells in a given sample that are within G_1/G_0, G_2, or S phase at the time of fixation, as well as to quantify cells in the sub-G_1 phase prior to apoptosis.

100 tests

NEW CFSE Cell Division Assay Kit 10009853

Stability: ≥1 year at -20°C
Summary: Carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) is a novel cell-tracing fluorescent dye used to examine the proliferative activity of cells by the labeling of a parent generation and the inheritance of the label by daughter generations. CFDA-SE diffuses into cells, where the acetate groups on the molecule are cleaved to yield a highly fluorescent derivative (CFSE) that is retained in the cell and can be detected by flow cytometry. Cell division results in sequential halving of fluorescence, and up to eight divisions can be monitored before the fluorescence is decreased to the background fluorescence of unstained cells. Cayman's CFSE Cell Division Assay provides an easy to use format for labeling and tracing cells through successive cell divisions which can be used to study the induction and inhibition of cell division in any *in vitro* model. The kit contains sufficient reagents for labeling and analyzing 100 cell samples by flow cytometry. CFSE can also be combined with any fluorochrome compatible with FITC for use in flow cytometry.

100 tests

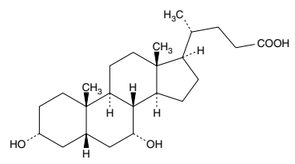


BDCM (a human DC-like cell line which can be obtained from ATCC) stimulates T cell proliferation when the cells are co-cultured together for six days. Human peripheral blood lymphocytes isolated from freshly collected blood were labeled with CFSE on Day 0. CFSE-labeled lymphocytes were then co-cultured with BDCM cells at a ratio of 25:1 in 2 ml of RPMI culture medium in a 6-well plate for three or six days. Panel A: CFSE fluorescence intensity is strong at the time of staining (Day 0). Panel B and C: CFSE staining intensity drops rapidly in the first couple of days due to catabolism. As cell division occurs, the staining intensity stabilized (Day 3 and Day 6). Panel C: Eight peaks representing successive cell cycles of lymphocytes were detected after six days of BDCM stimulation (the first peak shown here actually contains two peaks representing undivided cells, peak 0, and first division cells, peak 1).

Chenodeoxycholic Acid 10011286

[474-25-9] Anthropodeoxycholic Acid, CDCA, Fluibil, Hekbilin, Kebilis, Ulmenide
MF: $C_{24}H_{40}O_4$ **FW:** 392.6 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A bile acid and FXR ligand (EC_{50} = 13-34 µM) that is a key regulator of cholesterol homeostasis; exhibits toxicity that is linked to increased glutathione and increased oxidative stress; excess CDCA contributes to liver and intestinal cancers

1 g
5 g
10 g
25 g



3α,7α-dihydroxy-5β-cholan-24-oic acid

ChREBP DBD (human recombinant) 10009524

ChREBP DNA Binding Domain
M_r: 38.3 kDa **Purity:** ≥85% by SDS-Page
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride, 1 mM DTT, and 40% glycerol
Source: Recombinant GST-tagged ChREBP amino acids 648-741 expressed in *E. coli*

5 µg
10 µg
25 µg

•Also Available: **ChREBP DBD Western Ready Control** (10009753)

Chromosome Associated Protein-C Polyclonal Antibody (aa 47-61) 13503

CAP-C
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human CAP-C amino acids 47-61 • Host: rabbit • Cross Reactivity: (+) human CAP-C • Application(s): WB

1 ea

Chromosome Associated Protein-C Polyclonal Antibody (aa 281-297) 13501

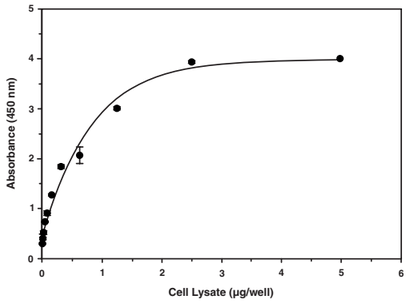
CAP-C
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human CAP-C amino acids 281-297 • Host: rabbit • Cross Reactivity: (+) human CAP-C • Application(s): WB

1 ea

ChREBP Transcription Factor Assay Kit 10006909

Stability: ≥6 months at -20°C
Summary: ChREBP is a transcription factor playing a critical role in the nutrient and hormonal regulation of genes encoding enzymes of glucose metabolism and lipogenesis pathways. Cayman's ChREBP Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A specific dsDNA sequence containing the ChREBP response element is immobilized onto the wells of a 96-well plate. ChREBP contained in a nuclear extract binds specifically to the ChREBP response element. ChREBP is detected by addition of specific primary antibody directed against ChREBP. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

96 wells



•Also Available: **ChREBP Cell-Based Translocation Assay Kit** (10010060)

CREB (Phospho-Ser[133]) Transcription Factor Assay Kit 10009846

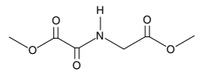
Stability: ≥6 months at -20°C
Summary: Cayman's CREB (Phospho-Ser[133]) Transcription Factor Assay is a non-radioactive, sensitive method for detecting CREB DNA binding activity. CREB contained in a nuclear extract or whole cell lysate binds specifically to the DNA cAMP response element immobilized to the wells of a 96-well plate. The activated CREB transcription factor complex is detected by addition of a specific primary antibody directed against Phospho-Ser[133] on CREB. A secondary antibody conjugated to HRP provides a sensitive colorimetric readout at 450 nm.

96 wells

Dimethyloxallyl Glycine 71210

[89464-63-1] DMOG
MF: $C_6H_9NO_5$ **FW:** 175.1 **Purity:** ≥98%
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: A cell permeable, competitive inhibitor of HIF-α prolyl hydroxylase; stabilizes HIF-1α expression at normal oxygen tensions in cultured cells at concentrations between 0.1 and 1 mM

10 mg
50 mg
100 mg
500 mg



N-(methoxyoxoacetyl)-glycine methyl ester

DNMT Antibodies

Antibody	Antigen	Cross Reactivity	Application	Supplied As
DNA Methyltransferase 1-Associated Protein 1 Polyclonal Antibody Catalog No. 13536	Amino acids 250-300 from human DMAP1 **Host:** rabbit	(+) chimpanzee, bovine, canine, human, murine, and rat DMAP1	IHC WB	Protein G-purified IgG
DNA Methyltransferase 1 Monoclonal Antibody (Clone 60B1220.1) Catalog No. 13479	Amino acids 637-650 from human DNMT1 **Host:** mouse, clone 60B1220.1	(+) human, murine, and zebrafish DNMT1	ChIP IHC (paraffin-embedded sections) IP WB	IgG
DNA Methyltransferase 2 Monoclonal Antibody (Clone 102B1259.2) Catalog No. 13481	Peptides corresponding to mouse DNMT2 **Host:** mouse, clone 102B1259.2	(+) human and murine DNMT2	WB	Protein G-purified IgG
DNA Methyltransferase 2 Polyclonal Antibody Catalog No. 13480	Amino acids 39-54 and 361-376 from murine DNMT2 **Host:** rabbit	(+) human and murine DNMT2	WB	Protein G-purified IgG
DNA Methyltransferase 3a Monoclonal Antibody (Clone 64B1446) Catalog No. 13484	Recombinant mouse DNMT3a **Host:** mouse, clone 64B1446	(+) human and murine DNMT3a	ChIP IF/ICC IHC (paraffin-embedded sections) WB	Protein G-purified IgG
DNA Methyltransferase 3a Monoclonal Antibody - Biotinylated (Clone 64B814.1) Catalog No. 13483	Recombinant mouse DNMT3a **Host:** mouse, clone 64B814.1	(+) human and murine DNMT3a	ELISA	Protein G-purified IgG
DNA Methyltransferase 3a Monoclonal Antibody (Clone 64B814.1) Catalog No. 13482	Recombinant mouse DNMT3a **Host:** mouse, clone 64B814.1	(+) human and murine DNMT3a	ICC/IF WB	Protein G-purified IgG
DNA Methyltransferase 3b Monoclonal Antibody (Clone 52A1018) Catalog No. 13485	Recombinant mouse DNMT3b **Host:** mouse, clone 52A1018	(+) human and murine DNMT3b	ChIP ICC IF IHC (paraffin-embedded sections) IP WB	Protein G-purified IgG

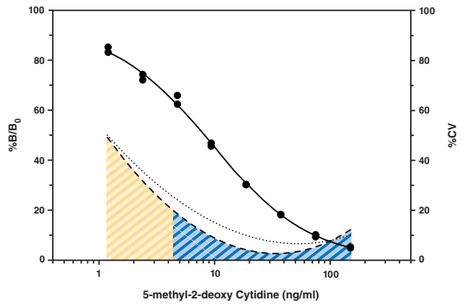
DNA Methylation EIA Kit 589324

Stability: ≥1 year at -20°C
Sensitivity: 50% B/B_0: 12 ng/ml • 80% B/B_0: 3 ng/ml
Specificity: DNA methylation is an important epigenetic process regulating gene expression. Methylation occurs on carbon 5 of 2-deoxy cytidine yielding the modified base 5-methyl-2-deoxy cytidine. The methylation pattern of cells is tightly regulated during development with the methylation profile being transmitted from parent to daughter cells during cell division. Methylation results in long-term silencing of genes, while unmethylated regions of DNA can be actively transcribed. Cayman's DNA Methylation EIA is a competitive assay that can be used for the quantification of 5-methyl-2-deoxy cytidine in urine, culture supernatants, plasma, and other sample matrices. The EIA typically displays IC_{50} (50% B/B_0) and IC_{80} (80% B/B_0) values of approximately 12 and 3 ng/ml, respectively.

5-methyl-2-deoxy Cytidine	100%
5-methyl Cytidine	20%
2-deoxy Cytidine	0.1%
Cytidine	0.1%

For a full specificity profile, please go to www.caymanchem.com

96 wells
480 wells

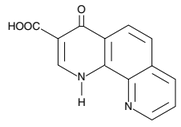


• Also Available: **DNA Methylation EIA Kit (Solid Plate)** (589325)

1,4-DPCA 71220

[331830-20-7] 1,4-dihydrophenonthrolin-4-one-3-Carboxylic Acid
MF: $C_{13}H_8N_2O_3$ **FW:** 240.2 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A competitive inhibitor of prolyl 4-hydroxylase (IC_{50} = 2.4-3.6 µM)

5 mg
10 mg
25 mg
50 mg

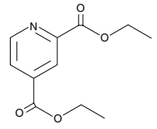


4,4-dihydro-4-oxo-1,10-phenanthroline-3-carboxylic acid

2,4-DPD 71200

[41438-38-4] 2,4-Diethylpyridine dicarboxylate
MF: $C_{11}H_{13}NO_4$ **FW:** 223.2 **Purity:** ≥98%
A soution in ethanol **Stability:** ≥1 year at -20°C
Summary: A cell permeable, competitive inhibitor of HIF- prolyl hydroxylase with effective concentrations in the low µM range

10 mg
25 mg
50 mg
100 mg

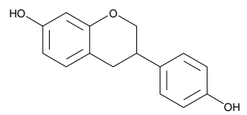


2,4-pyridinedicarboxylic acid, diethyl ester

(±)-Equol 13184

[94105-90-5]
MF: $C_{15}H_{14}O_3$ **FW:** 242.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A nonsteroidal estrogen produced from the metabolism of the isoflavonoid phytoestrogen daidzein by human intestinal microflora; exhibits EC_{50} values of 200 and 74 nM for human ERα and ERβ, respectively; induces breast cancer cell proliferation *in vitro* at concentrations as low as 100 nM

5 mg
10 mg
25 mg
50 mg

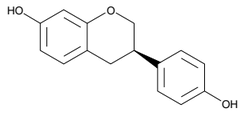


3,4-dihydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol

(R)-Equol 10010172

[221054-79-1] (+)-Equol, Isoequol
MF: $C_{15}H_{14}O_3$ **FW:** 242.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An ER agonist that binds to ERα and ERβ with K_i values of 27.4 and 15.4 nM, respectively; demonstrates higher ER agonist activity at ERα compared to ERβ (EC_{50} = 66 and 330 nM, respectively)

1 mg
5 mg
10 mg
25 mg

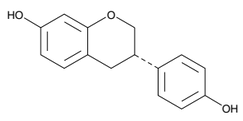


3,4-dihydro-3R-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol

(S)-Equol 10010173

[531-95-3] 4',7-Dihydroxyisoflavan, (-)-Equol, 4',7-Isoflavandiol
MF: $C_{15}H_{14}O_3$ **FW:** 242.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: The naturally occurring enantiomer of equol that demonstrates ER agonist activity similar to that of genistein (EC_{50} = 85 and 65 nM for human ERα and ERβ, respectively); preferentially binds ERβ (K_i = 0.73 nM) with lower affinity for ERα (K_i = 6.4 nM)

1 mg
5 mg
10 mg
25 mg

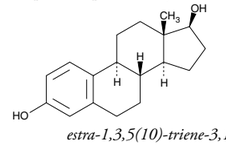


(S)-3,4-dihydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol

Estradiol 10006315

[50-28-2] β-Estradiol, 17β-Estradiol, 17β-Oestradiol
MF: $C_{18}H_{24}O_2$ **FW:** 272.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: The major estrogen secreted by the premenopausal ovary

500 mg
1 g
5 g
10 g

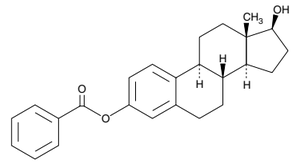


estra-1,3,5(10)-triene-3,17-diol

Estradiol Benzoate 10006487

[50-50-0] β-Estradiol benzoate, 17β-Estradiol 3-benzoate, 17β-Oestradiol benzoate
MF: $C_{25}H_{28}O_3$ **FW:** 376.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An estradiol analog that binds to ER with IC_{50} values in the range of 22-28 nM

100 mg
500 mg
1 g
5 g

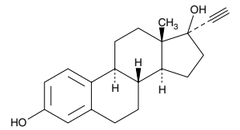


estra-1,3,5(10)-triene-3,17-diol 3-benzoate

Ethynyl Estradiol 10006486

[57-63-6]
MF: $C_{20}H_{24}O_2$ **FW:** 296.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A synthetic analog of estradiol used commonly as an oral contraceptive

100 mg
500 mg
1 g
5 g

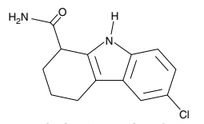


19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol

EX-527 10009798

[49843-98-3]
MF: $C_{13}H_{13}ClN_2O$ **FW:** 248.7 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A cell-permeable, selective inhibitor of SIRT1 (IC_{50} = 98 nM); inhibits other SIRTs only at much higher concentrations and has no effect on other HDACs

1 mg
5 mg
10 mg
25 mg



6-chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide

EZH1 Polyclonal Antibody 13487

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptides of human EZH1 • Host: rabbit • Cross Reactivity: (+) human and murine EZH1 • Application(s): WB • EZH1 is a human homolog of the *Drosophila* gene enhancer of zeste E(z), a member of the polycomb group of transcription factors.

1 ea

Formaldehyde Assay Kit 700380

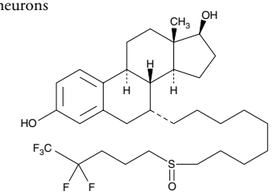
Stability: ≥6 months at 4°C
Summary: Formaldehyde (HCHO) is the most common aldehyde in the environment and is widely present in as a pollutant in water and air. Of importance to biomedical research, formaldehyde is a product of the demethylation reaction of FAD-containing oxidases, typified by LSD1, as well as the JmjC domain-containing enzymes, all of which play a key role in regulating the methylation status of numerous proteins and DNA. Cayman's Formaldehyde Assay is a fluorometric assay that can be used to quickly measure formaldehyde formation from enzymatic demethylation reactions, as well as in various biological and environmental samples. The assay is performed in a 96-well format with fluorescence detection using excitation and emission wavelengths of 370 and 470 nm, respectively.

96 wells

Fulvestrant 10011269

[129453-61-8] Faslodex®, ICI 182,780
MF: $C_{32}H_{47}F_5O_3S$ **FW:** 606.8 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent ER antagonist that works by both down-regulating and degrading ERα; efficacious in the treatment of estrogen-sensitive breast cancer; fully activates ER on hippocampal neurons

1 mg
5 mg
10 mg
50 mg

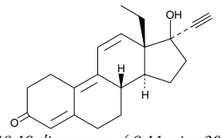


7α-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-estra-1,3,5(10)-triene-3,17β-diol

Gestrinone 10006488

[16320-04-0]
MF: $C_{21}H_{24}O_2$ **FW:** 308.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A synthetic steroid used occasionally to treat endometriosis; binds to androgen, and progesterone receptors with EC_{50} values of approximately 20 and 30 nM, respectively

100 mg
500 mg
1 g
5 g

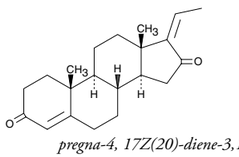


13-ethyl-17α-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one

NEW (Z)-Guggulsterone 71800

[39025-23-5]
MF: $C_{21}H_2O_2$ **FW:** 312.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A competitive antagonist of FXR (IC_{50} = 17 µM) that lowers LDL cholesterol and triglyceride levels in rodents fed a high cholesterol diet; demonstrates anti-tumor-promoting effects in human multiple myeloma and DU145 human prostate cancer cells

5 mg
10 mg
25 mg
50 mg



pregna-4, 17Z(20)-diene-3,16-dione

HAT Inhibitor Screening Assay Kit 10006515

Histone Acetyltransferase
Stability: ≥6 months at -20°C
Summary: Cayman's HAT Inhibitor Screening Assay provides a fast, fluorescence-based method for evaluating pCAF HAT inhibitors. The procedure requires only three easy steps, all performed in the same microwell plate. In the first step of the protocol, HAT is incubated with acetyl-CoA and a histone H3 peptide. During this time, HAT catalyzes the enzymatic transfer of acetyl groups from acetyl-CoA to the H3 peptide producing an acetylated peptide and CoASH. Following addition of isopropanol to stop the enzymatic reaction, CPM is added to the wells of the plate. CPM reacts with the free thiol groups present on CoASH forming a highly fluorescent product that is detected using excitation and emission wavelengths of 360-390 and 450-470 nm, respectively.

96 wells

vol. 8
Ep

Tom Brock, Ph.D.

Protein Acetylation:
Much More than Histone Acetylation

Just last decade, everyone was excited about the Human Genome Project, as well as all the other genome projects, and the gene was king. Today, epigenetics is reminding us of something that we already knew, that non-genetic factors are important in shaping gene expression and development. Similarly, where phosphorylation once seemed the primary way to modulate proteins, epigenetics has re-introduced us to acetylation as an important force in defining protein function. In particular, the acetylation of histones has moved to center stage, even though it was described over 45 years ago. Research on histone acetylation has led to a resurgence in the interest in enzymatically-mediated acetylation of other proteins. This article examines acetylation as a post-translational modification of proteins that impacts gene expression and plays a role in epigenetics.

The Basics

Acetylation refers to the addition of an acetyl group (CH_3CO) to organic compounds. Proteins can be acetylated by both enzymatic and non-enzymatic processes. One group of acetyltransferases commonly catalyze the transfer of an acetyl group from acetyl-CoA to the terminal amine on the side chain of lysine residues (Figure 1). These enzymes are commonly called HATs, because their best-known substrates have been histones. However, the nomenclature is being revised to lysine acetyltransferases (KATs), reflecting their ability to acetylate lysine (denoted 'K') on many proteins.[1] The KATs are numerous, with many assigned, based on structural similarities, to either the GNAT (Gcn5-related N-acetyltransferases) superfamily or the MYST (MOZ, YBF2/Sas3, Sas2, Tip60) family. Other important KATs include p300 (E1A-associated protein 300 kDa), CBP (cAMP response element binding (CREB)-binding protein), and TAFII 250 (TATA-binding protein associated factor II 250). The conversion of the positively charged lysine to acetyl-lysine, like the addition of negative phosphates to uncharged amino acids during phosphorylation, alters protein structure and interactions with other biomolecules. For example, acetylation of histones typically promotes the recruitment of effector proteins, relaxation of chromatin conformation, and an increase in transcription.

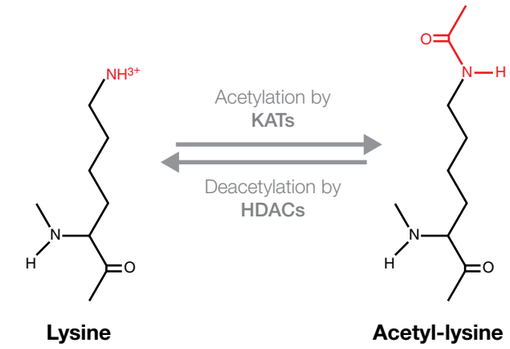


Figure 1. The enzymatic interconversion of lysine and acetyl-lysine

Like phosphorylation, acetylation is reversible. Histone deacetylases (HDACs, *a.k.a.* KDACs) are a smaller group of evolutionarily conserved enzymes. The human class I HDACs are homologous to the yeast enzyme Rpd3 and include HDAC1, 2, 3, and 8. Class II HDACs are homologous to yeast HDA1 and are divided into class IIa (HDAC4, 5, 7, 9) and class IIb (HDAC6 and 10) based on structure. The human class III HDACs include the sirtuin family of NAD^+-dependent protein deacetylases. The novel HDAC11 has a distinct structure and is a class IV HDAC. The HDACs often participate in the formation of transcriptional repressor complexes, inducing chromatin compaction through histone deacetylation, and silencing gene expression.

A Diversity of Partners

A great resource for the research scientist is the National Center for Biotechnology Information (NCBI), your tax dollars at work compiling information about everything molecular. This site should be your first stopping point when trying to learn authoritative information about a new protein or gene that you're studying. Information at this site helps to underscore two points about KATs and deacetylases: they are social enzymes, always interacting with other proteins, and they are promiscuous, binding to an astounding array of partners. Take, for example, the KAT known commonly as p300. At the NCBI gene link (http://www.ncbi.nlm.nih.gov/sites/entrez), entering 'human p300' finds the gene EP300 (KAT3B), with a summary stating that it associates with the adenovirus protein E1A, acetylates histones, binds CREB, and is a co-activator of HIF-1□ (hypoxia-inducible factor 1α). Further down, we find that it binds three different proteins produced by the lentivirus human immunodeficiency virus (HIV)-1. Then, impressively, is a list of over two hundred proteins that have been documented to directly interact with p300 (with links to references and other interactome datasets included). Similarly, the deacetylase HDAC1 is summarized as a histone deacetylase that also interacts with retinoblastoma tumor-suppressor to control cell growth and, together with metastasis-associated protein-2, deacetylates the tumor suppressor p53. Like p300, HDAC1 has an amazing list of partners: it interacts with some 300 proteins, with over 125 of these documented as direct binding partners.

The abundance of protein partners, for both KATs and HDACs, suggests that these enzymes tend to form multimeric complexes. In fact, such complexes serve the critical purpose of positioning the (de)acetylases at specific sites to perform their functions. Certainly, KATs can directly acetylate substrates *in vitro*. However, KAT activity *in vivo* is regulated, at least in part, by where it is positioned. For example, the classical model for activation of PPARs (peroxisome proliferator-activated receptors) posits that this receptor heterodimerizes at specific response elements with RXR (retinoid X receptor). In the absence of ligand, the unactivated heterodimer binds co-repressor proteins, such as nuclear receptor co-repressors (NCoR), G-protein pathways suppressor 2 (GPS2), and HDACs (Figure 2). The HDACs help prevent expression of PPAR-specific genes by keeping the neighboring histones deacetylated. The appearance of a ligand for PPAR causes dissociation of the co-repressor proteins followed by the recruitment of co-activators, including PPAR co-activator (PGC-1), CREB binding protein (CBP), and p300. Formation of the PPAR activation complex leads to histone acetylation by CBP and p300, giving rise to altered expression of genes involved in fatty acid metabolism, lipid homeostasis, and adipocyte differentiation. In this example, ligand binding to its receptor causes a large scale switch from a cluster of proteins serving various roles in preventing transcription to a different group designed to facilitate gene transcription.

Acetylation Patterns

In its simplest form acetylation is merely another form of post-translational modification of proteins. A good example is the acetylation of tubulin, which can be deacetylated by HDAC6 or SIRT2. Acetylation of this key microtubule component appears to alter its affinity for kinesin-1 and redirect motor-based trafficking of vesicles.[2,3] In short, acetylation changes protein function by adjusting protein-protein interactions. The net 'global' acetylation, in this case, may be determined by the balance of overall KAT and HDAC activities.

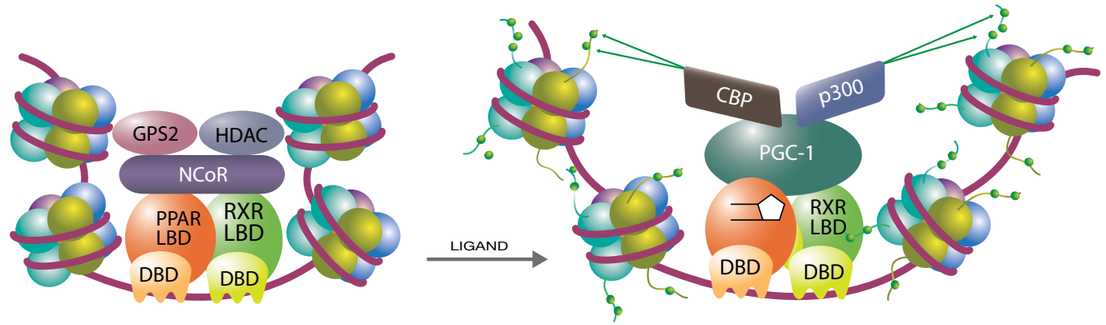


Figure 2. Binding of a PPAR ligand to the PPAR ligand binding domain (LBD) results in the release of co-repressor proteins, including NCoR, G-protein pathway suppressor 2 (GPS2), and histone deacetylase (HDAC), followed by the recruitment of PPAR co-activator (PGC-1), histone acetyltransferase p300, and CREB binding protein (CBP). Acetylation of histones by CBP and p300 relaxes chromatin, allowing transcription.

More commonly, acetylation is targeted to specific proteins and, possibly, specific lysine residues on those protein targets. One way that this can be achieved is by the formation of protein complexes containing either KATs or HDACs, as in the PPAR case described above. The assembly of the complex serves to place the KATs/HDACs near histones, transcription factors, or other targets. Histones, assembled as an octamer core surrounded by DNA, have amino termini that are freely exposed (Figure 3). Positively-charged lysine residues on these tails interact electrostatically with negatively-charged phosphate groups along the DNA backbone. Acetylation reduces these interactions and loosens the DNA, facilitating transcription. Bear in mind that, while it is generally true that histone acetylation increases transcriptional activation, there are exceptions. For example, acetylation of estrogen receptor-α suppresses ligand sensitivity and reduces ligand-induced transcriptional activity.[4,5]

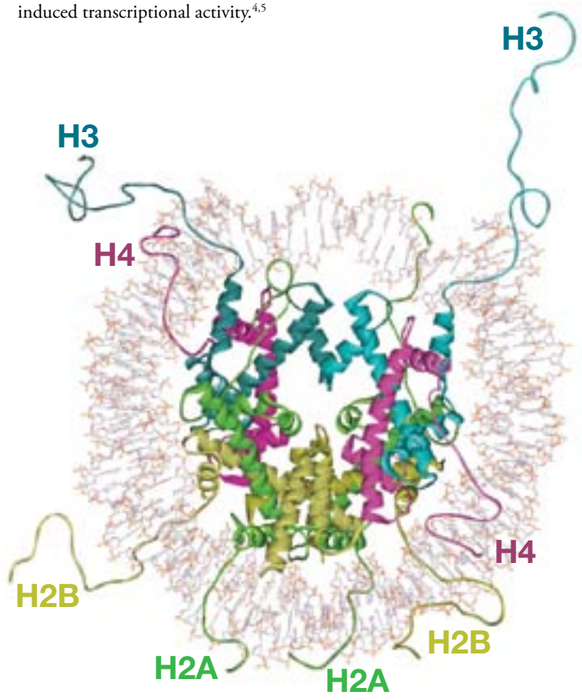


Figure 3. Nucleosomal structure, highlighting amino termini of histones projecting outward

In some cases, acetylation competes with other modifications.[6,7] For example, the tumor suppressor p53 contains a lysine-rich basic domain near its carboxy terminus. Six different lysine residues, spanning sites 370-386 on human p53, can be modified by acetylation, methylation, ubiquitination, neddylation, or sumoylation. In addition, serines that can be targeted for phosphorylation are interspersed amongst the lysines. It is clear that acetylation facilitates p53 activation, leading to gene expression that is relevant to p53's roles in responding to DNA damage and driving tumor suppression. At the other end of the response spectrum, ubiquitination of p53 targets it for degradation, preventing p53-mediated transcription and down-stream effects. Certain changes may predominate in the cytoplasm or in the nucleus, when p53 is associated with its negative regulator Mdm2, or when p53 monomers are forming homotetramers on gene-specific p53 response elements. For p53, acetylation may serve multiple roles, including stabilizing the protein, altering association with other proteins including other p53 monomers, enhancing its binding to DNA, and regulating transcription. By preventing ubiquitination, acetylation prevents the export and degradation of p53.

Acetylation and Epigenetics

While, strictly speaking, any mechanism for modifying gene expression (other than altering DNA sequence) constitutes an epigenetic change, the most interesting mechanisms are those that are long lasting. While acetylation marks can be readily removed by deacetylases, there are many ways to prolong acetylation. For example, positioning KATs in protein complexes next to specific targets may enable repeated acetylation of crucial residues, even if marks are spuriously removed. Levels of certain HDACs decline as cell differentiation progresses, reducing the rate of mark removal.[8,9] Certain HDACs may be directly inhibited, as DBC-1 (deleted in breast cancer-1) does to SIRT1,[10] preserving SIRT1-sensitive acetylation marks. Interestingly, acetylation of tubulin occurs on a site that is concealed following microtubule assembly,[2] physically preventing HDACs from access until the tubulin becomes exposed. As yet, it is not known if there are proteins that physically protect certain acetylated targets, as 14-3-3 isomers shield specific phosphorylated proteins. Certainly, these and other ways to extend (or decrease) acetylation half times remain to be discovered.

References
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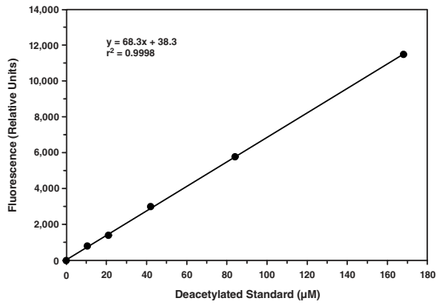
HDAC Activity Assay Kit 10011563

Stability: ≥6 months at -80°C
Summary: HDACs catalyze the hydrolytic removal of acetyl groups from histone lysine residues resulting in chromatin condensation and transcriptional repression of chromosomal DNA. Thus, HDAC inhibition allows the conformation of DNA to be relaxed and transcriptional activation to ensue. Cayman's HDAC Activity Assay provides a fast, fluorescent-based method for measuring Class I and II HDAC activity that eliminates radioactivity, extraction, or chromatography. The procedure requires only two easy steps, both performed in the same microplate. The fluorescent reaction product is easily analyzed using a plate reader with excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm.

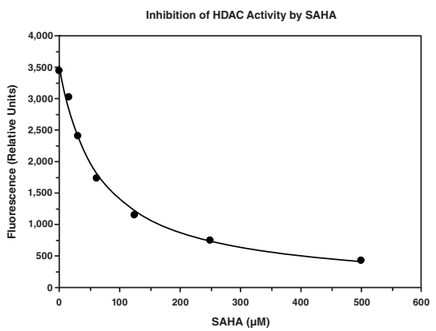
96 wells



HDAC Cell-Based Activity Assay Kit 600150

Stability: ≥6 months at -80°C
Summary: Cayman's HDAC Cell-Based Assay provides an easy tool for studying HDAC activity modulators in whole cells. By using a cell-permeable HDAC substrate, the activity of various protein lysine-specific deacetylases including HDAC1-containing complexes can be measured in intact cells in a simple and homogenous manner. The fluorescence of the deacetylated reaction product can be analyzed with excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm. An HDAC inhibitor, trichostatin A (TSA), is included for checking specificity of the HDAC reaction. This assay compliments Cayman's HDAC Activity Assay (Catalog No. 10011563), which uses a nuclear extract rather than whole cells for the assay. Together, both assays will help to identify whether an inhibitor/activator has a direct effect on the enzyme.

96 wells



HDAC1 (human recombinant) 10009231

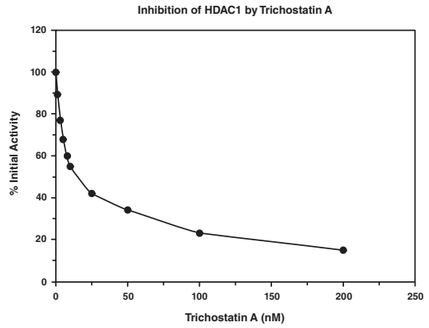
M_r: 79.9 kDa **Purity:** ≥10% by SDS-PAGE
Supplied as: 50 µg in 25 mM Tris-HCl, pH 8.0, containing 130 mM sodium chloride, 0.05% Tween 20, and 10% glycerol
Summary: Recombinant protein containing a C-terminal GST-tag expressed in *Sf*21 cells

1 ea

HDAC1 Inhibitor Screening Assay Kit 10011564

Stability: ≥6 months at -80°C
Summary: HDACs catalyze the hydrolytic removal of acetyl groups from histone lysine residues resulting in chromatin condensation and transcriptional repression of chromosomal DNA. Thus, HDAC inhibition allows the conformation of DNA to be relaxed and transcriptional activation to ensue. Cayman's HDAC1 Inhibitor Screening Assay provides a fast, fluorescent-based method for screening HDAC1 inhibitors. The procedure requires only two easy steps, both performed in the same microplate. The fluorescent reaction product is easily analyzed using a fluorometer with excitation wavelengths of 340-360 nm and emission wavelengths of 440-465 nm. Sufficient purified HDAC1 is provided for 100 tests.

96 wells



HDAC1 Polyclonal Antibody 13491

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptides corresponding to amino acids 1-5, 433-448, and 467-482 of human HDAC1 • Host: rabbit • Cross Reactivity: (+) human HDAC1 • Application(s): WB

1 ea

HDAC2 (human recombinant) 10009377

M_r: ~60 kDa **Purity:** ≥70%
Supplied as: 50 µg in 25 mM Tris-HCl, pH 8.0, containing 138 mM sodium chloride, 0.05% Tween 20, and 10% glycerol
Source: Full length recombinant protein containing a C-terminal His-tag expressed in *Sf*9 cells

1 ea

HDAC3 (human recombinant) 10009232

M_r: ~49.7 kDa **Purity:** ≥50%
Supplied as: 50 µg in 50 mM Tris-HCl, pH 8.0, containing 138 mM sodium chloride, 20 mM glutathione, and 10% glycerol
Source: Recombinant protein containing a complex of human HDAC3 with a C-terminal His-tag and human NCOR2 amino acids 395-489 with an N-terminal GST-tag

1 ea

HDAC3 Polyclonal Antibody 13493

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human HDAC3 amino acids 2-17 • Host: rabbit • Cross Reactivity: (+) human HDAC3 • Application(s): ChIP, IP, and WB

1 ea

HDAC4 Polyclonal Antibody 13494

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human HDAC4 amino acids 2-17 • Host: rabbit • Cross Reactivity: (+) human and murine HDAC4 • Application(s): ChIP, IP, and WB

1 ea

HDAC5 (human recombinant) 10009379

M_r: ~51 kDa **Purity:** ≥90% by SDS-PAGE
Supplied as: 5 µg in 25 mM Tris-HCl, pH 8.0, containing 138 mM sodium chloride, 0.05% Tween 20, and 10% glycerol
Source: Recombinant protein consisting of amino acids 657-1123 with a C-terminal His-tag expressed in *Sf*9 cells

1 ea

HDAC6 (human recombinant) 10009465

M_r: ~159 kDa **Purity:** ≥80%
Supplied as: 50 µg in 25 mM Tris-HCl, pH 8.0, containing 138 mM sodium chloride, 0.05% Tween 20, and 10% glycerol
Source: Recombinant protein with an N-terminal GST-tag expressed in *Sf*9 cells

1 ea

HDAC6 Polyclonal Antibody 13499

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human HDAC6 amino acids 1-16 • Host: rabbit • Cross Reactivity: (+) human and murine HDAC6 • Application(s): ChIP, IP, and WB

1 ea

HDAC7 (Phospho-Ser^{155}) Polyclonal Antibody 13500

Supplied as: Peptide-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human HDAC7 containing phospho-Ser^{155} • Host: rabbit • Cross Reactivity: (+) chimpanzee, bovine, canine, human, monkey, murine, and rat HDAC7 • Application(s): WB

1 ea

HDAC8 (human recombinant) 19380

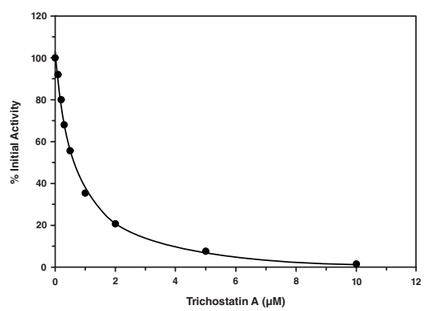
M_r: 45.3 kDa **Purity:** ≥95%
Supplied in: 10 mM Tris, pH 7.5, containing 100 mM sodium chloride, 3 mM MgCl2, and 20% glycerol
Source: Recombinant protein with a C-terminal His-tag expressed in *E. coli*

25 µg
50 µg
100 µg

HDAC8 Inhibitor Screening Assay Kit 700230

Stability: ≥6 months at -80°C
Summary: Human HDAC8 is a class I HDAC and has been identified in a variety of human cancer tissues. Cayman's HDAC8 Inhibitor Screening Assay provides a convenient fluorescence-based method for screening HDAC8 inhibitors. The procedure requires only two easy steps, both performed in the same microplate. In the first step, the substrate, which comprises the p53 sequence Arg-His-Lys-Lys(ε-acetyl)-AMC, is incubated with human recombinant HDAC8. Deacetylation sensitizes the substrate such that treatment with the developer in the second step releases a fluorescent product. Fluorescence is then analyzed with an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm.

96 wells



HDAC9 (human recombinant) 10009466

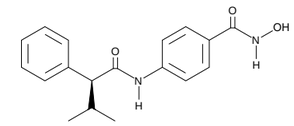
M_r: ~50.7 kDa **Purity:** ≥95%
Supplied as: 5 µg in 25 mM Tris-HCl, pH 8.0, containing 138 mM sodium chloride, 0.05% Tween 20 and 10% glycerol
Source: Recombinant protein consisting of amino acids 604-1,066 with a C-terminal His-tag expressed in *Sf*9 cells

1 ea

NEW (S)-HDAC-42 13277

[935881-37-1] AR42
MF: $C_{18}H_{20}N_2O_3$ **FW:** 312.4 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent inhibitor of HDACs (IC_{50} = 16 nM *in vitro*); decreases the viability of prostate cancer cell lines (IC_{50} = 0.40 µM); strongly suppresses the growth of PC-3 tumor xenografts

1 mg
5 mg
10 mg
25 mg



N-[4-[(hydroxyamino)carbonyl]phenyl]-αS-(1-methylethyl)-benzeneacetamide

HIF-1α (C-Term) Polyclonal Antibody 10006421

Supplied as: Peptide affinity-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: HIF-1α C-terminal amino acids 809-826 • Host: rabbit • Cross Reactivity: (+) human, murine, and simian HIF-1α • Application(s): (+) WB; (-) ICC and IP

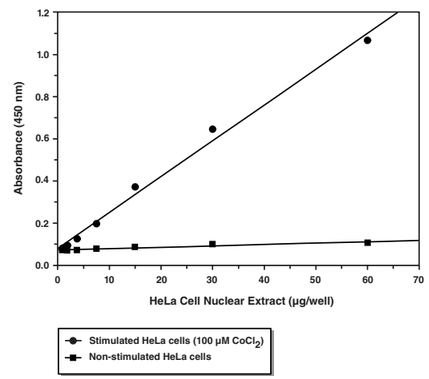
1 ea

• Also Available: **HIF-1α (C-Term) Blocking Peptide (300003)**

HIF-1 Transcription Factor Assay Kit 10006910

Stability: ≥6 months at -80°C
Summary: Cayman's HIF-1 Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A specific dsDNA consensus sequence containing the HIF-1 response element is immobilized to the wells of a 96-well plate. HIF-1 contained in a nuclear extract or whole cell lysate binds specifically to the HIF-1 response element. The HIF transcription factor complex is detected by addition of a specific primary antibody directed against HIF-1. A secondary antibody conjugated to HRP provides a sensitive colorimetric readout at 450 nm.

96 wells



HIF-2α Polyclonal Antibody 13505

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human HIF-2α amino acids 426-443 • Host: rabbit • Cross Reactivity: (+) human HIF-2α • Application(s): WB

1 ea

NEW Histone H2A (human recombinant) 10261

M_r: 14.2 kDa **Purity:** ≥85%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 300 mM sodium chloride, 1 mM DTT, 1 mM EDTA, and 50% glycerol
Source: Recombinant full length protein expressed in *E. coli*

50 µg
100 µg
250 µg

Histone H2A Polyclonal Antibody 13535

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human histone H2A amino acids 1-15 and 81-96 • Host: rabbit • Cross Reactivity: (+) human and murine histone H2A • Application(s): ELISA and WB

1 ea

NEW Histone H2B (human recombinant) 10262

M_r: 13.7 kDa **Purity:** ≥85%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 300 mM sodium chloride, 1 mM DTT, 1 mM EDTA, and 20% glycerol
Source: Recombinant full length protein expressed in *E. coli*

50 µg
100 µg
250 µg

Histone H2B (C-Term) Polyclonal Antibody 13538

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human histone H2B amino acids 111-125 • Host: rabbit • Cross Reactivity: (+) chicken, canine, *Drosophila*, human, most mammals, murine, rat, and zebrafish histone H2B • Application(s): WB

1 ea

Histone H2B (N-Term) Polyclonal Antibody 13539

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptides from human histone H2B • Host: rabbit • Cross Reactivity: (+) human histone H2B • Application(s): WB

1 ea

NEW Histone H3 (human recombinant) 10263

M_r: 15.5 **Purity:** ≥85%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride, 1 mM DTT, 1 mM EDTA, and 20% glycerol
Source: Recombinant full length protein expressed in *E. coli*

50 µg
100 µg
250 µg

Histone H3 (Phospho-Ser[28]) Monoclonal Antibody (Clone 117C826) 13540

PHH3
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human histone H3 • Host: mouse, clone 117C826 • Cross Reactivity: (+) human histone H3 • Application(s): WB

1 ea

NEW Histone H4 (human recombinant) 10264

M_r: 11.5 kDa **Purity:** ≥85%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride, 1 mM DTT, 1 mM EDTA, and 20% glycerol
Source: Recombinant full length protein expressed in *E. coli*

50 µg
100 µg
250 µg

Histone H4 Polyclonal Antibody 13543

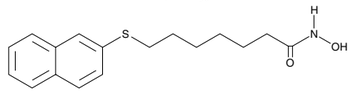
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human histone H4 amino acids 15-30 • Host: rabbit • Cross Reactivity: (+) human histone H4 • Application(s): WB

1 ea

HNHA 13295

[926908-04-5] HDAC Inhibitor VI
MF: $C_{17}H_{21}NO_2S$ **FW:** 303.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A cell-permeable inhibitor of HDAC activity (IC_{50} = 100 nM)

5 mg
10 mg
25 mg
50 mg



N-hydroxy-7-(2-naphthalenylthio)-heptanamide

Hsf1 Monoclonal Antibody (Clone 10H8) 10011433

Heat Shock Factor 1
Supplied as: IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: rat Hsf1 • Host: rat, clone 10H8 • Isotype: IgG_1 • Cross Reactivity: (+) human, murine, rat, bovine, guinea pig, hamster, monkey, and rabbit Hsf1 • Application(s): ELISA, ICC, IP, and WB

25 µg
100 µg

Hsf2 Monoclonal Antibody (Clone 3E2) 10011434

Heat Shock Factor 2
Supplied as: IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: purified mouse recombinant Hsf2 • Isotype: IgG_1 • Host: rat, clone 3E2 • Cross Reactivity: (+) human, murine, rat, guinea pig, hamster, monkey, rabbit, canine, bovine, ovine, and porcine Hsf2 • Application(s): WB

25 µg
100 µg

NEW JMJD2A (human recombinant) 10336

Jumonji Domain Containing 2A, Lysine (K)-specific Demethylase 4A
M_r: 43.0 kDa **Purity:** Clarified Lysate
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant protein expressed in *E. coli*

25 Units
50 Units
100 Units

NEW JMJD2D (human recombinant) 10335

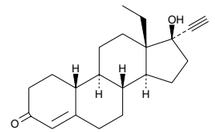
Jumonji Domain Containing 2D, Lysine (K)-specific Demethylase 4D
M_r: 42.7 kDa **Purity:** Clarified Lysate
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant protein expressed in *E. coli*

25 Units
50 Units
100 Units

Levonorgestrel 10006318

[797-63-7] Norplant
MF: $C_{21}H_{28}O_2$ **FW:** 312.5 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A synthetic progesterone analog (*i.e.,* a progestin) and the biologically active component of norgestrel, which is a racemic mixture

100 mg
500 mg
1 g
5 g

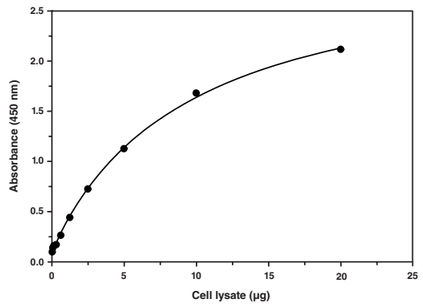


13-ethyl-17β-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one

Liver X Receptor β Transcription Factor Assay Kit 10011119

Stability: ≥1 year at -80°C
Summary: LXRs are ligand-activated transcription factors that are primarily activated by oxysterols and cholesterol metabolites. As such, LXRs play an important role in the regulation of cholesterol, lipid, and carbohydrate metabolism. There are two known isoforms of LXR: LXRα and LXRβ. LXRβ is ubiquitously expressed in all tissues while LXRα is primarily expressed in the liver, adipose tissue, small intestine, and macrophages. LXRs are currently being examined as potential therapeutic targets in the treatment of diabetes, cardiovascular disease, Alzheimers disease, obesity, and atherosclerosis. Cayman's LXRβ Transcription Factor Assay is a sensitive colorimetric method for detecting specific transcription factor binding activity from nuclear exctracts and whole cell lysates in a 96-well format.

96 wells



LSD1 (human recombinant) 10245

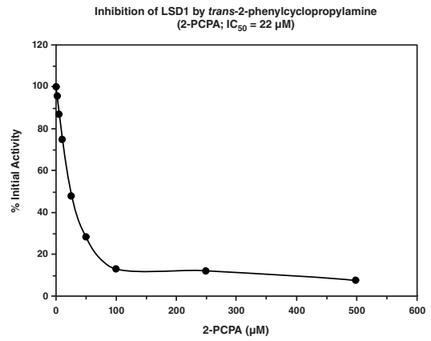
AOF2, BHC110, KDM1, NPAO, p110b
M_r: 94 kDa **Purity:** >50%
Supplied in: 50 mM sodium phosphate, pH 7.2 containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal His-tagged enzyme expressed in *E. coli*

25 Unit
50 Unit
100 Unit

LSD1 Inhibitor Screening Assay Kit 700120

Stability: ≥6 months at -80°C
Summary: LSD1 is a histone demethylase whose actions on specific lysine residues alter transcription of chromosomal DNA. It also inhibits the tumor suppressor activity of p53 by demethylating a specific lysine residue. Inhibitors of LSD1 are important tools used to elucidate mechanisms of transcription and cell cycle progression and have therapeutic potential for treating cancer. Cayman's LSD1 Inhibitor Screening Assay provides a convenient fluorescence-based method for screening LSD1-specific inhibitors. The assay is based on the multistep enzymatic reaction in which LSD1 first produces H_2O_2 during the demethylation of lysine 4 on a peptide corresponding to the first 21 amino acids of the N-terminal tail of histone H3. In the presence of horseradish peroxidase, H_2O_2 reacts with ADHP to produce the highly fluorescent compound resorufin that can be analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

96 wells



LSD1 Polyclonal Antibody (aa 100-150) 13554

Amine Oxidase (flavin containing) Domain 2
Supplied as: Peptide affinity-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide corresponding to a portion of human LSD1 amino acids 100-150 • Host: rabbit • Cross Reactivity: (+) canine, human, murine, rat, Rhesus monkey, and zebrafish LSD1 • Application(s): WB

1 ea

LSD1 Polyclonal Antibody (aa 400-450) 13553

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide corresponding to a portion of human LSD1 amino acids 400-450 • Host: rabbit • Cross Reactivity: (+) chimpanzee, bovine, canine, human, monkey, and murine LSD1 • Application(s): WB

1 ea

LSD1 Polyclonal Antibody (aa 450-500) 13486

Supplied as: Protein A-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide within the region of human LSD1 amino acids 450-500 • Host: rabbit • Cross Reactivity: (+) chimpanzee, bovine, canine, equine, human, murine, orangutan, and porcine LSD1 • Application(s): WB

1 ea

LSD1 Polyclonal Antibody (aa 800-850) 13555

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human LSD1 with the range of amino acids 800-850 • Host: rabbit • Cross Reactivity: (+) canine, human, murine, rat, and Rhesus monkey LSD1 • Application(s): IHC (paraffin-embedded sections) and WB

1 ea

vol. 8
Ep

Olivia May, Ph.D.

DNA Methylation:
Fingerprints of the (epi)genome

Methylation of DNA is an integral epigenetic component of cellular development and differentiation as well as a basis for a number of human diseases. Accomplished by the transfer of a methyl group from S-adenosylmethionine (SAM) to the 5-carbon position of the pyrimidine ring of cytosine (Figure 1), DNA methylation contributes to the epigenome by covalently modifying the structure of DNA. Methylated 5' cytosine was serendipitously discovered as an extraneous (fifth?) base over 60 years ago while R. D. Hotchkiss was sorting and quantifying DNA bases from nuclear preparations. It functions to both temporally and spatially regulate gene transcription. Patterns of DNA methylation are specific to each cell and tissue type and thus act, much like a fingerprint, as a means of identification. Once these epigenetic marks have been established, they are faithfully propagated over many cell generations.

CpG Islands and Shores: Potential Sites of Methylation

Over evolutionary time, the sequence CpG (cytosine linked with a phosphodiester bond to guanine) has been progressively eliminated from the genome due to deamination of methylcytosines to thymines, leaving in its wake long stretches of DNA without these alternating repeats. A CpG island is defined as a sequence with a GC content that is greater than 55% and ratio of CpG to GpC of at least 0.65.[1,2] CpG islands are at least 500 base pairs long and are located within 5' promoter regions of genes. In order for a gene to be expressed, stretches of CpG sequences in the promoter regions of genes must remain unmethylated. Due to CG suppression, the frequency of CpG dinucleotide repeats occurring in the CpG-poor 'shores' outside promoters in the coding region of the gene is relatively low (~1%). However, unlike CpG islands in promoters, 70% of CpG dinucleotide repeats located in the coding region of the gene are methylated and eventually deaminated to become thymine. Furthermore, different CpG sites are methylated in different tissues, resulting in a pattern of methylation that is gene and tissue specific. This unique pattern of methylation confers upon the genome specific cell-type identity, and thus plays a central role in cellular differentiation and development.

Getting to a Fifth Base

Eukaryotic DNA methyltransferases (DNMTs) operate within the 5'-CG-3' dinucleotide to establish a pattern of methylation by catalytically removing the methyl group from SAM (which becomes S-adenosylhomocysteine (SAH)) and transferring it to the 5-carbon of cytosine. DNMTs also maintain the status of CpG methylation after replication ensuring it will be heritable in future generations. Three active mammalian DNMTs, DNMT1, DNMT3a, and DNMT3b, display separate preferences for distinct methylation events. DNMT3a and DNMT3b act as functional enzymes during early development playing a role in *de novo* DNA methylation. Despite being catalytically inactive, DNMT3L is required for establishing genomic imprints and regulates DNMT3a and DNMT3b by stimulating their catalytic activity. DNMT1 preferentially adds methyl groups to hemi-methylated DNA (only one of the double stands) taking on the role of maintaining the methylation pattern once it is established. This so called maintenance methylation occurs in the replication complex where DNMT1 recognizes the normally methylated CpG sites in the parent strand and catalyzes the addition of a methyl group in the corresponding CpG site of the daughter strand. This type of methylation contributes to the inheritance of DNA methylation marks over generations of replication. Both classes of DNMTs can however participate in either form of methylation and there is direct evidence of interaction among DNTM1, 3a, and 3b *in vivo*. A third class, DNMT2, displays weak DNA methyltransferase catalytic activity. Recent evidence indicates that DNMT2 methylates cytosine 38 in the anticodon loop of aspartic acid transfer RNA.[3] Many more DNMTs exist in prokaryotes that are associated with DNA restriction-modifications systems.

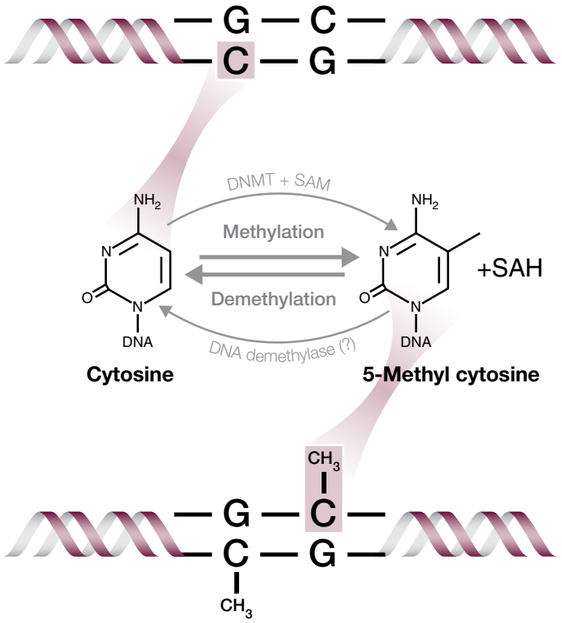


Figure 1. Methylation modification of DNA at the 5-carbon position of cytosine by DNMTs, where SAM donates the $-CH_3$ group and is converted to SAH. This reaction is potentially reversible by a yet to be defined DNA demethylase.

Chromatin Structure Determines Gene Expression

DNA methylation status is closely linked with chromatin structure. Regions of euchromatin that actively enable gene expression are associated with hypomethylated DNA; inactive, condensed heterochromatin contains hypermethylated DNA. CpG islands are normally unmethylated in actively transcribed genes and require protection against hypermethylation. Methylation of gene promoters interferes with the binding of certain transcription factors and can attract methylated DNA-binding proteins that in turn recruit other modifying enzymes leading to a chromatin configuration that is unfavorable to gene expression (see below). Thus, DNA methylation is an effective means by which gene expression is silenced. It also serves to suppress repetitive sequences and transposon errors, thus enhancing genome stability. In normal cells, DNA methylation functions to prevent the expression of imprinted and inactive X chromosome genes. In cancerous cells, DNA methylation inactivates tumor-suppressor genes, as well as DNA repair genes, can disrupt cell-cycle regulation, and activates (*via* hypomethylation) certain oncogenes. Managing to therapeutically regulate methylation in instances such as these has enormous potential for the prevention and treatment of human cancers.

Cross Talk with Histones

Modifications of histones associated with chromatin can directly and indirectly affect the establishment of DNA methylation patterns. Conversely, DNA methylation influences histone modifications. Hypermethylation of CpG islands within gene promoters triggers deacetylation of local histones. Methyl-CpG-sequence binding domain (MBD) proteins such as MeCP2 selectively bind to methylated regions of DNA *via* an MBD and recruit histone deacetylases (HDAC). This results in a deacetylated, repressive chromatin structure that prohibits transcription factor binding. HDAC

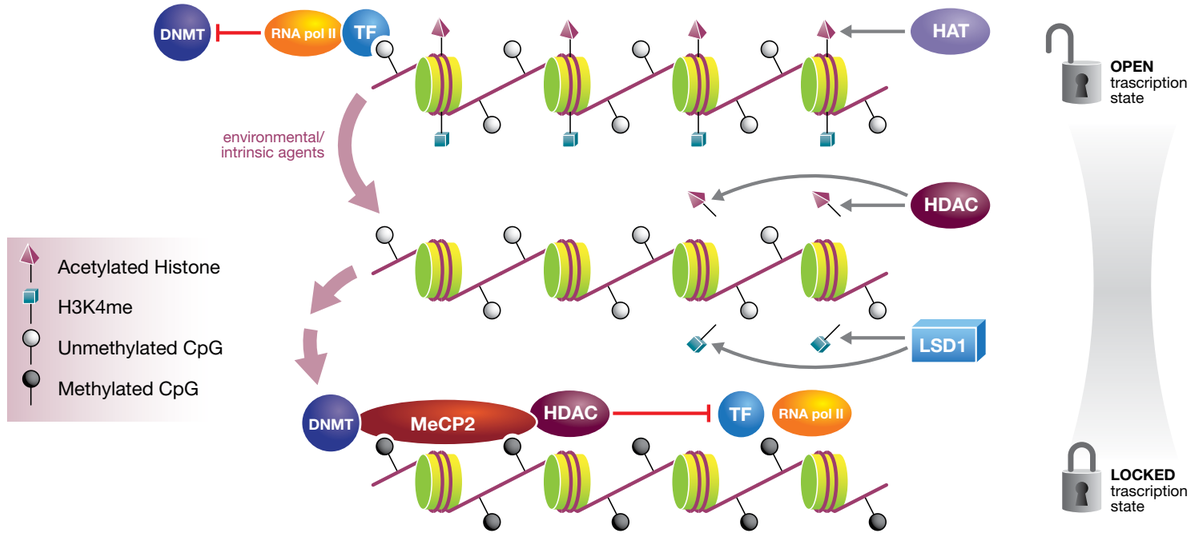


Figure 2. Schematic model of events relating DNA methylation to gene transcription. A permissive state for transcription includes histones acetylated by HAT as well as methylated at H3K4 (H3K4me). Unmethylated CpGs are bound by transcription factors and RNA polymerase II thereby blocking interaction with DNMT. Environmental influences potentially trigger reversal of acetylation by HDAC and removal of methylation by LSD1. In this state, CpGs are vulnerable to methylation by DNMT and are bound by MeCP2, which recruits HDAC. HDAC maintains a deacetylated state of the histone, locking the chromatin in a repressed state that prohibits transcription factor binding. Presently the precise order of these events is unclear.

inhibitors, which restore histone acetylation, contribute to the removal of MeCP2 from methylated cytosines, allowing HATs to reacetylate histones at the promoter; thus HAT activity restores conditions favorable to gene transcription. Clearly cross talk occurs between histone acetylation and DNA methylation. Which event triggers the other is still open for debate. Current consensus seems to favor DNA methylation as secondary to the process of gene silencing with hypoacetylation of histones initiating a closed chromatin state (Figure 2). Hypoacetylated chromatin may be recognized by *de novo* DNMTs that methylate vulnerable CpG sites, which would lock the gene promoter in a repressive state.

Besides acetylation, other chromatin modifications such as histone methylation can direct DNA methylation and *vice versa*. *de novo* DNA methylation has been associated with the removal of methyl groups from histone H3 lysine 4 (H3K4) by the histone demethylase, LSD1. H3K4 methylation (H3K4me) marks most CpG islands within gene promoters and is thought to protect them from *de novo* DNA methylation by recruiting transcription factors (such as Sp1 and CTCF). These transcription factors along with RNA polymerase II are hypothesized to block DNMT3a/b interaction with sites of transcriptional initiation. Also recently, LSD1 has been shown to directly demethylate and stabilize DNMT1, which is methylated by the histone methylase SET7/9.[4]

Methylation Reversal?

While a dynamic, reversible DNA methylation pattern has been proposed to be involved in memory formation in the brain[5] and as well in regulating a transcriptionally active promoter of an estrogen-induced gene,[6] there is controversy as to whether an active DNA demethylation process occurs in mammals. Although many enzymes have been proposed to act as specific DNA demethylases, compelling evidence to prove their mechanism of action remains lacking and wrought with contradictions.[7] The removal of existing methylation could possibly be obtained by passive demethylation, which occurs when DNMT1 fails to maintain the existing methylation pattern during replication or through nucleotide/base excision repair. Mechanical inhibition of methylation can occur by blocking the enzyme active site with RG-108 (a non-nucleoside DNMT inhibitor) or incorporating azanucleosides, such as 5-azacytidine or 5-aza-2'-deoxycytidine (AzadC), into the DNA. AzadC has been shown to restore the active state of the promoter by inducing histone acetylation.[8]

Regulation of gene transcription involves the intricate coordination of histone acetylation/deacetylation and histone methylation/demethylation activities with DNA methyltransferases. Many precise details of these interactions remain unknown. Cayman offers a variety of DNA methyltransferase antibodies as well as DNA methylation/methyltransferase detection kits to aid in the further study of these important epigenetic events.

References
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2. Takai, D. and Jones, P.A. *Proc. Natl. Acad. Sci. USA* **99**, 3740–3745 (2002).
3. Tovy, A., Tov, R.S., Gaentzsch, R., *et al. PLoS Pathog.* **6**, e1000775 (2010).
4. Pradhan, S., Chin, H.G., Estève, P.O., *et al. Epigenetic.* **4**, 383-387 (2009).
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6. Metivier, R., Gallais, R., and Tiffoche, C. *Nature* **452**, 45-50 (2008).
7. Ooi, S.K.T. and Bestor, T.H. *Cell* **133**, 1145-1148 (2008).
8. Patra, S.K. and Bettuzzi, S. *Biochemistry (Mosc)* **74**, 613-9 (2009).

Further Reading Recommended
Kondo, Y. and Yonsei *Med. J.* **50**, 455-463 (2009).
Vaissière, T., Sawan, C., and Herceg, Z. *Mutat. Res.* **659**, 40-48 (2008).
Ikegami, K., Ohgane, J., Tanaka, S., *et al. Int. J. Dev. Biol.* **53**, 203-214 (2009).

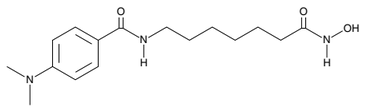
Related Products available from Cayman

Cat. No.	Product Name
589324	DNA Methylation EIA Kit
700140	Methyltransferase Colorimetric Assay Kit
700150	Methyltransferase Fluorometric Assay Kit
700270	SET7/9 Methyltransferase Inhibitor Screening Assay Kit
13302	RG-108 - DNMT Inhibitor
13536	DNA Methyltransferase 1-Associated Protein 1 Polyclonal Antibody
13479	DNA Methyltransferase 1 Monoclonal Antibody (Clone 60B1220.1)
13481	DNA Methyltransferase 2 Monoclonal Antibody (Clone 102B1259.2)
13480	DNA Methyltransferase 2 Polyclonal Antibody
13483	DNA Methyltransferase 3a Monoclonal Antibody - Biotinylated (Clone 64B814.1)
13484	DNA Methyltransferase 3a Monoclonal Antibody (Clone 64B1446)
13482	DNA Methyltransferase 3a Monoclonal Antibody (Clone 64B814.1)
13485	DNA methyltransferase 3b Monoclonal Antibody (Clone 52A1018)

M 344 — 13174

[251456-60-7] D237, Histone Deacetylase Inhibitor III, MS 344
MF: $C_{16}H_{25}N_3O_3$ **FW:** 307.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of HDACs, inhibiting maize HDAC (IC_{50} = 100 nM) as well as human HDAC1 (IC_{50} = 46 nM); shows a 3-fold selectivity for HDAC6 over HDAC1

5 mg
10 mg
25 mg
50 mg



4-(dimethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl]-benzamide

Methylated Lysine Polyclonal Antibody — 13727

Supplied as: Rabbit antiserum **Stability:** ≥1 year at -20°C
Summary: Antigen: methylated KLH • Host: rabbit • Cross Reactivity: (+) methylated lysine residues • Application(s): ELISA, IP, and WB

400 µl

Methylated Lysine Polyclonal Antibody-biotin — 13728

Supplied as: Rabbit immunoglobulin **Stability:** ≥1 year at -20°C
Summary: Antigen: methylated KLH • Host: rabbit • Cross Reactivity: (+) methylated lysine residues • Application(s): ELISA, IP, and WB

400 µl

Methylated Lysine Polyclonal Antibody HRP Conjugate — 13729

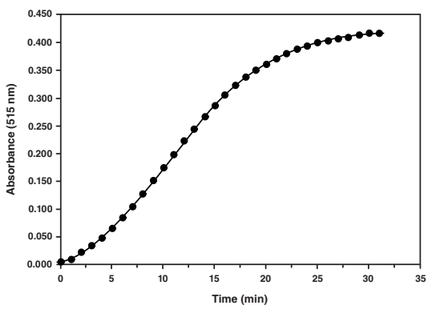
Supplied as: Rabbit antiserum **Stability:** ≥1 year at -20°C
Summary: Antigen: methylated KLH • Host: rabbit • Cross Reactivity: (+) multi-species • Application(s): ELISA and WB

400 µl

Methyltransferase Colorimetric Assay Kit — 700140

Stability: ≥6 months at -80°C
Summary: Methylation of key biological molecules and proteins plays important roles in numerous biological systems, including signal transduction, biosynthesis, protein repair, gene silencing, and chromatin regulation. The SAM dependent MTs use SAM as the enzymatic cofactor. SAM, also known as AdoMet, acts as a donor of a methyl group that is required for the modification of proteins and DNA. Cayman's MT Colorimetric Assay is a continuous enzyme-coupled assay that can continuously monitor SAM-dependent MT activities. The removal of the methyl group from SAM generates S-adenosylhomocysteine (AdoHcy), which is rapidly converted to urate and H_2O_2 by an enzyme mixture provided in the kit. The rate of production of H_2O_2 is measured with the colorimetric reagent, 3,5-dichloro-2-hydroxybenzenesulfonic acid, by an increase in absorbance at 500-520 nm. The assay is supplied with AdoHcy as a positive control. The assay can be used with any purified SAM-dependent MT.

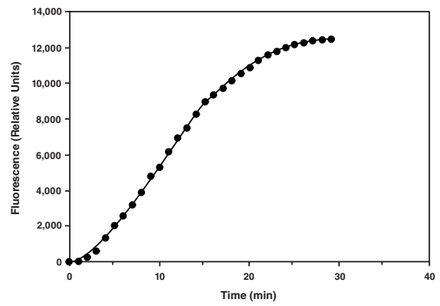
96 wells



Methyltransferase Fluorometric Assay Kit — 700150

Stability: ≥6 months at -80°C
Summary: Cayman's MT Fluorometric Assay is a continuous enzyme-coupled assay that can continuously monitor SAM-dependent MTs. The removal of the methyl group from SAM generates S-adenosylhomocysteine (AdoHcy), which is rapidly converted to urate and H_2O_2 by an enzyme mixture provided in the kit. The reaction between H_2O_2 and ADHP (10-acetyl-3,7,-dihydroxyphenoxazine) produces the highly fluorescent compound resorufin, which is analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. The assay is supplied with AdoHcy as a positive control. The assay can be used with any purified SAM-dependent MT.

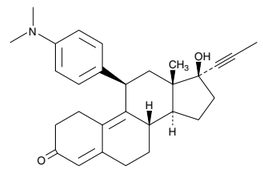
96 wells



Mifepristone — 10006317

[84371-65-3] RU-486
MF: $C_{29}H_{35}NO_2$ **FW:** 429.6 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent progesterone receptor and glucocorticoid receptor antagonist with K_i value of approximately 1 nM

100 mg
500 mg
1 g
5 g



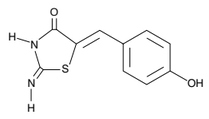
11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one

• Also Available: **Mifepristone-d_3** (10010660)

Mirin — 13208

[299953-00-7]
MF: $C_{10}H_8N_2O_2S$ **FW:** 220.3 **Purity:** ≥95%
An orange crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of the DNA damage sensor MRN, inhibiting MRN-dependent phosphorylation of histone H2AX (IC_{50} = 66 µM); prevents activation of ATM by blocking the nuclease activity of Mre11; induces G_2 arrest, abolishes the radiation-induced G_2/M checkpoint, and prevents homology-directed repair of DNA damage

5 mg
10 mg
50 mg
100 mg

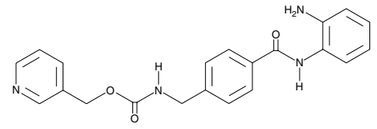


2-amino-5-[(4-hydroxyphenyl)methylene]-4(5H)-thiazolone

MS-275 — 13284

[209783-80-2] Entinostat, SNDX 275
MF: $C_{21}H_{20}N_4O_3$ **FW:** 376.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of HDACs that preferentially inhibits HDAC1 (IC_{50} = 300 µM) over HDAC3 (IC_{50} = 8 µM); does not inhibit HDAC8; induces p21/C1P1/WAF1, slowing cell growth, differentiation, and tumor development *in vivo*

1 mg
5 mg
10 mg
25 mg

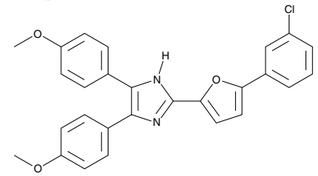


N-[[4-[[(2-aminophenyl)amino]carbonyl]phenyl]methyl]-3-pyridinylmethyl ester, carbamic acid

Neurodazine — 13224

[937807-66-4]
MF: $C_{27}H_{21}ClN_2O_3$ **FW:** 456.9 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: Induces neuronal differentiation in skeletal muscle cells, as indicated by the upregulated expression of neuron-specific markers; effective with mature muscle fibers as well as myoblasts

5 mg
10 mg
25 mg
50 mg



2-[5-(3-chlorophenyl)-2-furanyl]-4,5-bis(4-methoxyphenyl)-1H-imidazole

NF-κB (p50) (human recombinant) — 10009818

M_r: 74.5 kBa **Purity:** ≥75%
Supplied in: PBS, pH 7.4, containing 5 mM DTT and 20% glycerol
Source: Recombinant GST-tagged protein expressed in *E. coli*

5 µg
10 µg
25 µg

• Also Available: **NF-κB (p50) (human recombinant) Western Ready Control** (10010184)

NEW NF-κB (p50) Monoclonal Antibody — 13755

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: a portion of amino acids 150-200 of human NF-κB (p50) • Host: mouse, clone 2J10D7 • Isotype: $IgG_{1κ}$ • Cross Reactivity: (+) human NF-κB (p50) • Application(s): IHC and WB

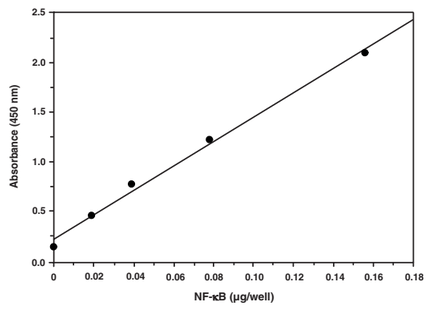
1 ea

• Also Available: **NF-κB (p50) NLS Inhibitory Peptide Set** (13760)

NF-κB (human p50) Transcription Factor Assay Kit — 10006912

Stability: ≥6 months at -20°C
Summary: Cayman's NF-κB (human p50) Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates in a 96-well ELISA format. Cayman's NF-κB (human p50) Transcription Factor Assay detects human NF-κB (p50). It will not cross-react with NF-κB (p65).

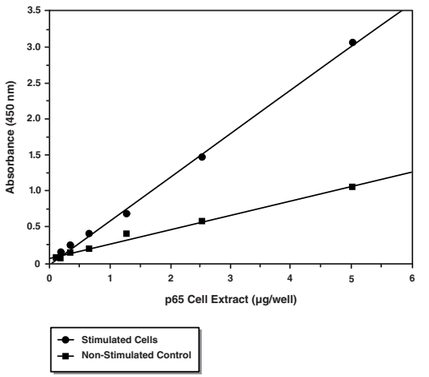
96 wells



NF-κB (human p50/p65) Combo Transcription Factor Assay Kit — 10011223

Stability: ≥6 months at -80°C
Summary: Cayman's NF-κB (human p50/p65) Combo Transcription Factor Assay is a non-radioactive, sensitive method for detecting p50 and p65 transcription factor DNA binding activity in nuclear extracts.

96 wells



NEW NF-κB (p65) Monoclonal Antibody — 13752

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human NF-κB (p65) amino acids 526-539 • Host: mouse, clone 112A1021 • Isotype: $IgG_{1κ}$ • Cross Reactivity: (+) human, murine, and rat NF-κB (p65) • Application(s): FC, IHC, and WB

1 ea

• Also Available: **NF-κB (p65) (Ser^{279}) Inhibitory Peptide Set** (13758)
NF-κB (p65) ($Ser^{529,536}$) Inhibitory Peptide Set (13759)

NEW NF-κB (p65) Monoclonal Antibody-biotin 13756

Supplied as: Protein G-purified IgG **Stability:** ≥6 months at 4°C
Summary: Antigen: human NF-κB (p65) amino acids 526-539 • Host: mouse, clone 112A1021 • Isotype: $IgG_{1κ}$ • Cross Reactivity: (+) human, murine, and rat NF-κB (p65) • Application(s): ELISA

1 ea

NEW NF-κB (p65) Polyclonal Antibody (aa 2-17) 13757

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human NF-κB (p65) amino acids 2-17 • Host: rabbit • Cross Reactivity: (+) chimpanzee, human, and monkey NF-κB (p65) • Application(s): WB

1 ea

NEW NF-κB (p65) NLS Polyclonal Antibody 13751

NF-κB (p65) Nuclear Localization Signal
Supplied as: Peptide affinity-purified **Stability:** ≥1 year at -20°C
Summary: Antigen: a portion of the NF-κB (p65) NLS • Host: rabbit • Cross Reactivity: (+) bovine, chimpanzee, gorilla, equine, human, monkey, and murine NF-κB (p65) • Application(s): ICC and WB

1 ea

NEW NF-κB (p65) Polyclonal Antibody (aa 538-546) 13753

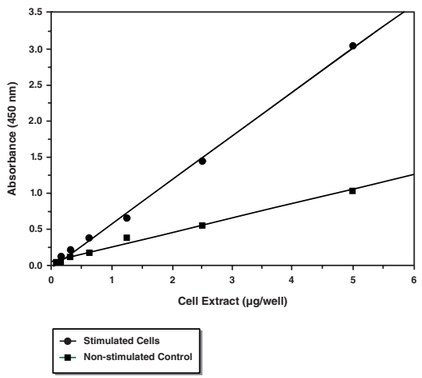
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human NF-κB (p65) amino acids 538-546 • Host: rabbit • Cross Reactivity: (+) human, murine, and rat NF-κB (p65) • Application(s): WB

1 ea

NF-κB (p65) Transcription Factor Assay Kit 10007889

Stability: ≥6 months at -20°C
Summary: Cayman's NF-κB (p65) Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates in a 96-well ELISA format. Cayman's NF-κB (p65) Transcription Factor Assay detects human NF-κB (p65). It will not cross-react with NF-κB (p50).

96 wells



Norgestrel 10006319

[6533-00-2] Ovrette®
MF: $C_{21}H_{28}O_2$ **FW:** 312.5 **Purity:** ≥90%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A synthetic progesterone analog (*i.e.*, a progestin) used as an oral contraceptive

100 mg
500 mg
1 g
5 g

(±)-13-ethyl-17[]-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one

NU 7026 13308

[154447-35-5] DNA-PK Inhibitor II, LY293646
MF: $C_{17}H_{15}NO_3$ **FW:** 281.3 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A cell-permeable, potent, specific, and ATP-competitive inhibitor of DNA-PK (IC_{50} = 230 nM); poorly inhibits PI3K (IC_{50} = 13 µM) and is inactive against ATM, ATR, and PARP-1

5 mg
10 mg
25 mg
50 mg

2-(4-morpholinyl)-4H-naphtho[1,2-b]pyran-4-one

(±)-Nutlin-3 10004372

[548472-68-0]
MF: $C_{30}H_{30}Cl_2N_4O_4$ **FW:** 581.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of p53-Mdm2 interaction (IC_{50} = 0.09 µM); induces the expression of p53-regulated genes and exhibits potent antiproliferative activity in cells with functional p53

1 mg
5 mg
10 mg
50 mg

NOTE: Sold under license from Hoffman-La Roche
(±)-4-[4,5-bis-(4-chlorophenyl)-2-(2-isopropoxy-4-methoxy-phenyl)-4,5-dihydro-imidazole-1-carbonyl]-piperazin-2-one

(+)-Nutlin-3 10009816

Nutlin 3b
MF: $C_{30}H_{30}Cl_2N_4O_4$ **FW:** 581.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inactive enantiomer of nutlin-3 that may serve as a useful control for non-Mdm2 related cellular activities; also called 'enantiomer b' based on the elution pattern during chiral separation of (±)-nutlin-3

500 µg
1 mg
5 mg
10 mg

NOTE: Sold under license from Hoffman-La Roche
4-(4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1H-imidazole-1-carbonyl)piperazin-2-one

(-)-Nutlin-3 18585

Nutlin 3a
MF: $C_{30}H_{30}Cl_2N_4O_4$ **FW:** 581.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent inhibitor of Mdm2-p53 binding (IC_{50} = 0.09 µM); induces the expression of p53-regulated genes and exhibits potent antiproliferative activity in cells with functional p53; also called 'enantiomer a' based on the elution pattern during chiral separation of (±)-nutlin-3

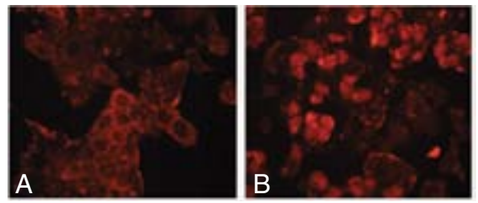
1 mg
5 mg
10 mg
25 mg

NOTE: Sold under license from Hoffman-La Roche
4-(4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydro-1H-imidazole-1-carbonyl)piperazin-2-one

p53 Cell-Based Activation/Translocation Assay Kit 600008

Stability: ≥6 months at -20°C
Summary: The tumor suppressor protein p53 plays a crucial role in coordinating cellular responses to genotoxic stress and holds many important clinical implications in the treatment of cancer. Cayman's p53 Cell-Based Activation/Translocation Assay provides a highly specific p53 primary monoclonal antibody together with a DyLight™ (product of Thermo Fisher Scientific) conjugated secondary antibody in a ready-to-use format. (-)-Nutlin-3, a potent inhibitor of Mdm2-p53 interaction, which has been shown by scientists at Cayman to cause the activation and translocation of p53 between the cytoplasm and nuclear compartments, is included as a positive control.

96 wells

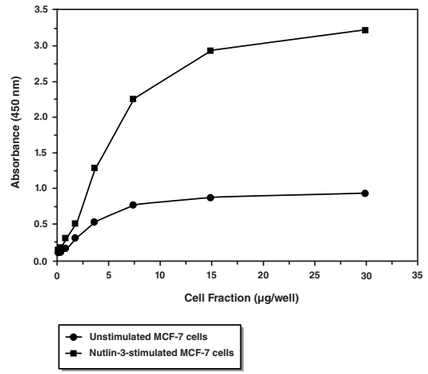


(-)-Nutlin-3-induced translocation of p53 in MCF-7 cells. *Panel A*: MCF-7 cells were treated with vehicle or *Panel B*: 50 µM (-)-Nutlin-3 for four hours, then fixed and stained with p53 monoclonal antibody according to the protocol described in the booklet. Translocation of p53 from cytoplasm to nuclei upon stimulation by (-)-Nutlin-3 is evident.

p53 Designer Transcription Factor Assay Kit 600030

Stability: ≥1 year at -80°C
Summary: Cayman's p53 Designer Transcription Factor Assay is designed to study alternate p53 DNA-binding sites. A biotinylated oligonucleotide is incubated with p53 contained in a nuclear extract; this mixture then binds to the streptavidin-coated plate provided in the kit. p53 is detected by addition of a specific primary antibody directed against p53. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

96 wells



p53-PAK 10005291

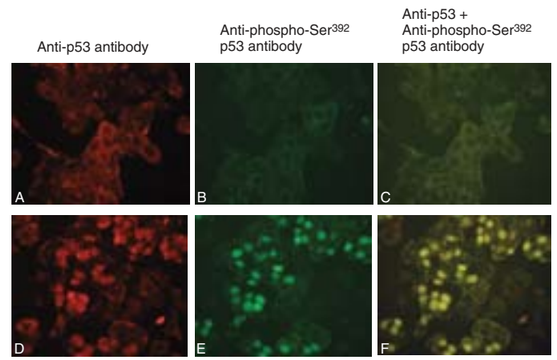
Stability: ≥1 year at -20°C
Summary: Contains PRIMA-1, p53 (Phospho-Ser[392]) polyclonal antibody, (±)-nutlin-3, and caylin-2

1 ea

p53 Total and p53 (Phospho-Ser[392]) Dual Staining Assay Kit 600060

Stability: ≥6 months at -20°C
Summary: Cayman's p53 Total and p53 (Phospho-Ser[392]) Dual Staining Assay Kit provides a pair of highly specific antibodies against total and phospho-p53 (Phospho-Ser[392]) together with a pair of matched DyLight™ (product of Thermo Fisher Scientific) conjugated secondary antibodies in a ready-to-use format. (-)-Nutlin-3, a potent inhibitor of Mdm2-p53 interaction which has been shown to cause the activation and translocation of p53 between the cytoplasm and nuclear compartments, is included as a positive control.

96 wells

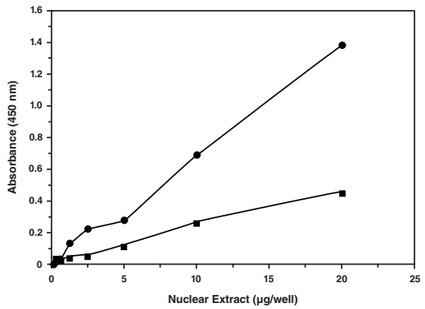


(-)-Nutlin-3-induced translocation of p53 in MCF-7 cells. MCF-7 cells were treated with vehicle (top panels) or 50 µM (-)-Nutlin-3 (bottom panels) for four hours, then fixed and stained as described in the assay protocol. Panel A and B shows that in unstimulated MCF-7 cells, most of p53 was not phosphorylated and appeared as cytoplasmic staining (strong staining of total protein in A and weak staining of phosphorylated protein in B). Panel C is the merged image of A and B. In contrast, panel D and E shows that upon stimulation by (-)-Nutlin-3, most of p53 was phosphorylated and appeared in the nucleus (strong staining of both total protein and phosphorylated protein in both D and E, respectively). Panel F is the merged image of D and E.

p53 Transcription Factor Assay Kit 600020

Stability: ≥1 year at -80°C
Summary: Cayman's p53 Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts. A specific dsDNA sequence containing the p53 response element is immobilized onto the wells of a 96-well plate. p53 contained in a nuclear extract binds specifically to the p53 response element and is detected by addition of a specific primary antibody directed against p53. A secondary antibody conjugated to HRP provides a sensitive colorimetric readout at 450 nm.

96 wells



PARP (Cleaved) Monoclonal Antibody 13557

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide containing amino acids near the 214/215 cleavage site of human PARP • Isotype: IgG2b • Host: mouse • Cross Reactivity: (+) human PARP • Application(s): FC (intracellular) and WB

1 ea

pCAF Histone Acetyltransferase 10009115

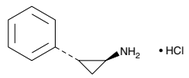
HAT, p300/(CREB binding protein) Associated Factor
M_r: ~40 kDa **Purity:** ≥95%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride, 1 mM EDTA, and 20% glycerol **Stability:** ≥6 months at -80°C
Source: Recombinant GST-tagged protein purified from *E. coli*

25 µg
50 µg
100 µg

2-PCPA (hydrochloride) 10010494

trans-2-Phenylcyclopropylamine (hydrochloride), Tranylcypromine (hydrochloride)
MF: $C_9H_{11}N$ • HCl **FW:** 169.7 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An irreversible, mechanism-based inhibitor of LSD1 with an IC_{50} value of 20.7 µM and a K_i value of 242.7 µM that effectively inhibits histone demethylation *in vivo*; irreversibly inhibits monoamine oxidases (MAO) A and MAO B with IC_{50} values of 2.3 and 0.95 µM and K_i values of 101.9 and 16 µM, respectively

10 mg
50 mg
100 mg
250 mg

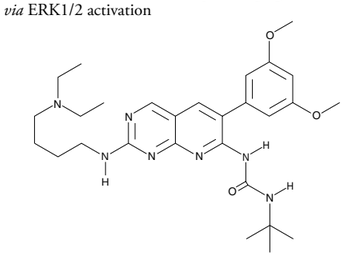


(1R,2S)-rel-2-phenyl-cyclopropanamine, monohydrochloride

PD 173074 13032

[219580-11-7]
MF: $C_{28}H_{41}N_7O_3$ **FW:** 523.7 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent, selective inhibitor of FGFR tyrosine kinase activity, blocking autophosphorylation of FGFR1 (IC_{50} = 21.5 nM); impairs angiogenesis, as well as self-renewal of stem cells *via* ERK1/2 activation

500 µg
1 mg
5 mg
10 mg

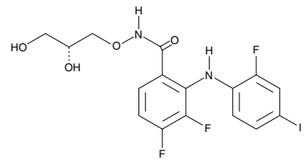


N-[2-[[4-(diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-d] pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea

PD 0325901 13034

[391210-10-9]
MF: $C_{16}H_{14}F_3IN_2O_4$ **FW:** 482.2 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent MEK inhibitor that suppresses phosphorylation of ERK in murine colon 26 tumors with an IC_{50} value of 0.33 nM; suppression of ERK activation with 1 µM PD 0325901 combined with 3 µM CHIR99021 (a glycogen synthase kinase-3 inhibitor) prevents cell differentiation and sustains self renewal of murine embryonic stem cells for at least eight passages

500 µg
1 mg
5 mg
10 mg

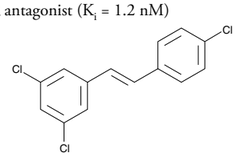


N-[(2R)-2,3-dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide

PDM 2 10006342

[688348-25-6]
MF: $C_{14}H_9Cl_3$ **FW:** 283.6 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent and selective AhR antagonist (K_i = 1.2 nM)

10 mg
50 mg
100 mg
500 mg

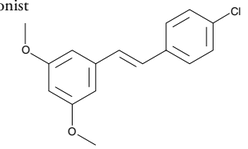


1,3-dichloro-5-[(1E)-2-(4-chlorophenyl)ethenyl]-benzene

PDM 11 10006341

MF: $C_{16}H_{15}ClO_2$ **FW:** 274.7 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A structural analog of several resveratrol derivatives which act as a potent and selective AhR antagonists and agonist

10 mg
25 mg
50 mg
100 mg

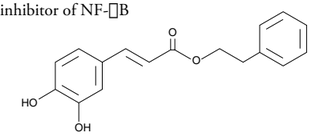


(E)-5-[2-(4-chlorophenyl)ethenyl]-1,3-dimethoxyphenyl

Phenethyl Caffeiate 70750

[115610-29-2] Caffeic Acid Phenethyl Ester, CAPE
MF: $C_{17}H_{16}O_4$ **FW:** 284.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: A potent and specific inhibitor of NF-κB

50 mg
100 mg
500 mg
1 g

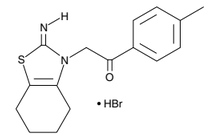


(E)-3-(3,4-dihydroxyphenyl)-2-propenoic acid, 2-phenylethyl ester

Pifithrin-α 13326

[63208-82-2]
MF: $C_{16}H_{18}N_2OS$ • HBr **FW:** 367.3 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inactivator of p53 that blocks p53-dependent transcriptional activation and apoptosis, preventing p53-mediated apoptosis by cytotoxic compounds in C8 cells at 10 µM and in human umbilical vein endothelial cells at 30 µM

5 mg
10 mg
25 mg
50 mg

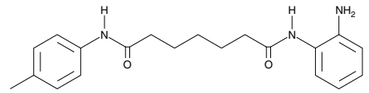


1-(4-methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)-ethanone, monohydrobromide

Pimelic Diphenylamide 106 13212

[937039-45-7]
MF: $C_{20}H_{25}N_3O_2$ **FW:** 339.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A slow, tight-binding inhibitor of class I HDACs, progressively binding HDACs and remaining bound after wash-out; inhibits class I HDACs (IC_{50} = 150, 760, 370, and 5,000 nM for HDAC1, 2, 3, and 8, respectively) but not class II HDACs (IC_{50} >180 µM for HDAC4, 5, and 7)

1 mg
5 mg
10 mg
25 mg



N1-(2-aminophenyl)-N7-(4-methylphenyl)-heptanediamide

PPARα LBD (human recombinant) 10009088

PPARα Ligand Binding Domain
M_r: ~34 kDa **Purity:** ≥90%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 20% glycerol, 100 mM sodium chloride, and 1 mM DTT
Source: Recombinant His-tagged protein expressed in *E. coli*

25 µg
50 µg
100 µg

PPARα Polyclonal Antibody 101710

Supplied as: Peptide affinity-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human, mouse, and rat PPARα amino acids 22-36 • Host: rabbit • Cross Reactivity: (+) human, murine, rat, ovine, and porcine PPARα; (-) PPARγ • Application(s): WB

1 ea

• Also Available: **PPARα Blocking Peptide** (301710)

PPAR Transcription Factor Assay Kits

PPARs are ligand-activated transcription factors belonging to the large superfamily of nuclear receptors. PPARα primarily activates genes encoding proteins involved in fatty acid oxidation, while PPARβ primarily activates genes directly involved in lipogenic pathways and insulin signaling. Members of the PPAR family are important direct targets of many antidiabetic and hypolipidemic drugs. Cayman's PPAR Transcription Factor Assays are a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A specific dsDNA sequence containing the PPAR response element is immobilized onto the bottom of wells of a 96-well plate. PPARs contained in a nuclear extract, bind specifically to the PPAR response element. PPARα, β, or γ are detected by addition of specific primary antibodies directed against the individual PPARs. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

PPARα, δ, γ Complete Transcription Factor Assay Kit 10008878

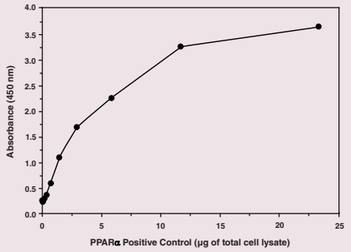
Stability: ≥1 year at -20°C
Summary: This kit contains individual primary antibodies for PPARα, β, and, γ to follow detection of each receptor in separate wells of the plate.

96 wells

PPARα Transcription Factor Assay Kit 10006915

Stability: ≥6 months at -20°C

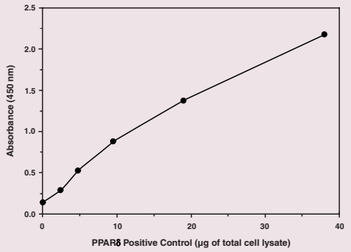
96 wells



PPARδ Transcription Factor Assay Kit 10006914

Stability: ≥6 months at -20°C

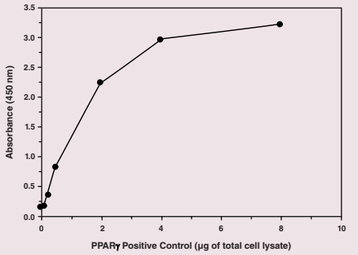
96 wells



PPARγ Transcription Factor Assay Kit 10006855

Stability: ≥6 months at -20°C

96 wells



vol. 8 Ep

Tom Brock, Ph.D.

Sex, Immortality, and Genetic Memory

Epigenetics is an exciting new field that is, well, incredibly complicated and, actually, not so new, when you get right down to it. The promise appears to be that, if we can come to understand the intricacies of epigenetics, then we can do great things for mankind. Perhaps more importantly, epigenetics has the potential to thrill us by providing hot chatter and, potentially, explaining great mysteries, mysteries like why women need men to reproduce and why humans need sex in the first place, the key to immortality, and how genes remember.

Sexy Imprints

Some of my best friends are eutherians. This, in itself, makes them very interesting, because imprinting in mammals is limited to eutherians. At least, imprinting of the genome is a eutherian characteristic. Psychology majors and animal trainers may know that some newborn birds behaviorally imprint on parents (or parent impersonators), like the cranes that were imprinted on ultralight planes and led along their migratory pathways. However, some scientists know that genomic imprinting, the epigenetic silencing of select genes in a parent-of-origin pattern, is a feature of placental mammals (eutherians), as well as plants and some insects. In this type of imprinting, only one allele is expressed, due to chemical modifications that persistently silence the other allele in somatic cells throughout normal development. As a result, the concepts of dominant/recessive alleles, homo/heterozygosity, and Mendelian inheritance are irrelevant to imprinted genes.

In humans, there are some 60 known (geneimprint.com) and 150 predicted imprinted genes.[1] Generally, all imprinting signals, as well as other genomic methyl marks, are stripped from primordial germ cells. In male spermatogenesis, imprint marks are re-established very early in development, while this process is delayed in oogenesis.[2] In both sperm and egg, global genomic methylation occurs during maturation, presumably to shut down transcription as the gametes switch to using stored mRNA for protein synthesis. Remarkably, after fertilization, sperm

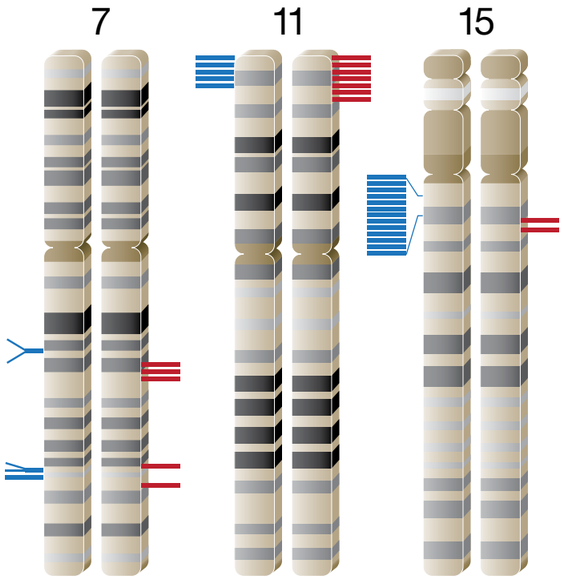


Figure 1. Clustering of imprinted genes on chromosomes 7, 11, and 15
Blue: paternally-expressed
Red: maternally-expressed

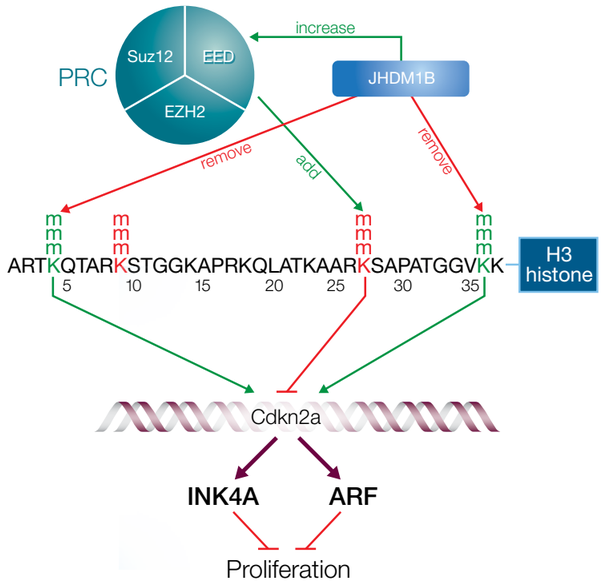


Figure 2. Histone methyltransferases and demethylases act together to modulate the expression of the growth suppressors produced by Cdkn2a, INK4A and ARF.

DNA is rapidly and actively demethylated, followed by demethylation of egg DNA. However, imprinted genes elude demethylation in the fertilized egg. As imprint marks are established in gametes, they are gender specific. The profile of imprinted genes are remarkably consistent for a given sex and species. However, those found in people are very different from those in mice.

In humans, the majority of imprinted genes are on three chromosomes (7, 11, and 15). They tend to be clustered relatively closely together (Figure 1). In some cases, they overlap on complementary strands, as for the paternally-expressed genes for sarcoglycan (SGCE, sense) and a zinc finger protein (PEG10, antisense) on chromosome 7. The genes that are expressed only on paternal chromosomes are completely distinct from those expressed only on maternal chromosomes. As a result, offspring must contain both maternal and paternal chromosomes, or survive without dozens of genes. About 50% more of the known imprinted genes occur on paternal chromosomes (35) than on maternal (23).

Several recent studies have suggested that errors in imprinting are associated with assisted reproductive technologies, which includes superovulation and *in vitro* fertilization.[3] It is argued that, because there is only a single expressed copy of imprinted genes, defects in these genes may more frequently contribute to pathologies. This thought gains force when one considers the types of products of imprinted genes. Paternally-expressed genes encode a half dozen zinc finger proteins, another half dozen non-coding or antisense mRNAs, several C/D box small nucleolar RNA (snoRNA), insulin, insulin-like growth factor, and several other proteins. Maternally-expressed genes encode one zinc finger protein, two non-coding RNAs, and no snoRNAs, but several ion transporters or channels and transcription factors, as well as co-factors, binding proteins, inhibitors, and binding proteins. It is not hard to imagine that altered expression of many of these gene products could have major effects.

Cellular Immortality

To the cell biologist, proliferation and differentiation are at opposite ends of a spectrum: the more you get of one, the less of the other. Cancer researchers might focus, instead, on cell growth and apoptosis as the key cellular fates. Some epigeneticists deal with nothing less than immortality, as opposed to cellular senescence. Senescence is marked by the irreversible cessation of cell division. This can be associated with telomere shortening, triggered by DNA damage, or developmentally programmed. Immortality, on the other hand, means unlimited cell proliferation, featured in stem cells and cancer cells alike.

One focal point of control in determining the holy grail of cell immortality is the gene for cyclin-dependent kinase inhibitor 2A (Cdkn2a, Figure 2). This gene produces two products, the 16 kDa inhibitor of cyclin-dependent kinase 4, p16 INK4A, and the 19 kDa alternative open reading frame variant, p19ARF. As a result, the gene is also referred to as INK4A/ARF. The gene products are important tumor suppressors, as they literally stop key enzymes that drive proliferation. Mutation of this locus is among the most frequent cytogenetic events that are associated with human cancer.[4]

The picture, in the healthy adult, is clear: the majority of cells are not supposed to divide, so they need abundant expression at the Cdkn2a locus. At the other end of the spectrum, inhibited expression at Cdkn2a, lies immortality. The methylation of histones plays a central role in determining expression. Generally, the effect of histone methylation depends on the methylation site. Methylation on histone 3 at lysine 9 and 27 (H3K9, H3K27) and on histone 4 at lysine 20 (H4K20) usually results in transcriptional repression, while methylation at H3K4, H3K36, and H3K79 produces transcriptional activation. Stem cell proliferation is stimulated by methylation of H3K27 through the action of the aptly-named Enhancer of zeste, homolog 2 (EZH2). This H3K27-specific methyltransferase forms the catalytic core, with proteins that include EED and Suz12, of a polycomb repressive complex (PRC). When active, this complex represses Cdkn2a expression, allowing cell proliferation (Figure 2).

The demethylases, remarkably, can augment this action. The jumonji domain-containing histone demethylase 1B (JHDM1B, also known as KDM2A) specifically removes methyl marks from H3K4 and H3K36.[5] Like lifting one's foot from the gas pedal, this slows transcription at the Cdkn2a locus, reducing the production of the inhibitors of cell cycling. Moreover, JHDM1B also indirectly increases the transcription of EZH2, stimulating methylation of H3K27, applying a brake to transcription of Cdkn2a. Through these actions, a sort of immortality is achieved.

Genetic Memory

Is anything forever? Can anything truly be immortal on a planet that might end in 2012 (Mayan date) or be uninhabitable in 2.3 billion years?[6] In a similar way, how stable is our genome? Australopithecines, like Lucy and Ardi, walked the earth just 3 to 4 million years ago, and neanderthals were foraging in Eurasia 100,000 years ago. Certainly, something better than *Homo sapiens* is likely to emerge in the (relatively) near future. This suggests that DNA, itself, is constantly changing, adapting, taking a shape that bears witness to the need to adjust to evolving conditions. Offspring certainly resemble their parents and even their grandparents, attesting to the persistence of genotype for a few generations. However, the regular shuffling of the DNA deck during sexual reproduction, combined with an inherent error rate in DNA duplication during mitosis and a varying rate of mutation, indicates that our DNA is but a faint memory of our ancestors and their experiences.

In a similar way, epigenetic marks are limited in their persistence. The most stable marks, DNA methyl groups, are completely erased during meiosis, only to be reestablished shortly after. While there are DNA methyltransferases that are dedicated to maintaining these marks on cytosines, newly synthesized DNA strands are produced with unmethylated cytosine bases. This means that marks must be actively re-established following DNA replication. Conceptually, there must be mechanisms in place to faithfully reproduce methyl marks on new DNA, as well as checking, correcting, and repairing processes akin to those that work on DNA. New experiences, stresses, and signals trigger the placement of new marks, and there must be ways to decide whether to preserve these 'memories' by reproducing the marks following DNA replication. Thus, DNA methylation appears to be a way to adjust gene expression in a relatively stable way, long-term. Importantly, these adjustments can still be reversed, either by removing or simply not renewing marks.

If DNA methylation provides long-term memory to gene expression, then histone modification might represent short-term memory. The variety of enzymes that attach or remove marks suggests that acetylation and methylation may be used in many different systems to produce a relatively temporary change in gene expression in specific ways. For example, the histone deacetylase HDAC2 participates in a cluster of proteins that acts as a functional model to maintain embryonic stem cell pluripotency.[7] HDAC2, combined with Nanog, Oct4, and other interacting proteins (Figure 3), work in concert to mediate transcriptional repression that defines embryonic stem cells.

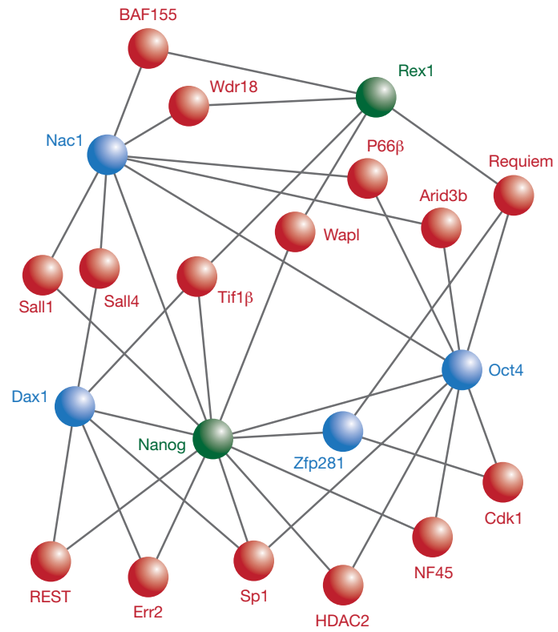


Figure 3. A protein interactome from embryonic stem cells
Stem cell marker proteins Nanog and Rex1 (green) were used to pull down interacting proteins, including core (blue) and peripheral (red) targets. *Adapted from reference 7.*

The beauty of histone modification is its flexibility. It can help a cell 'remember' that it is a stem cell for many cell cycles. Alternatively, specific signals can lead to a wholesale switch in how histones are marked, initiating cell differentiation.[8,9] Changed histone marks serve to preserve the memory of the new cues while at the same time altering DNA utilization in the production of an intermediate blast-type cell. When the cell finally becomes, say, a FoxP3+ T cell, histone marks will not only play a central role in defining gene expression, they will be telltale clues to the cell's history.

References
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PPAR Agonists and Antagonists

Product Name	PPARα (IC_{50} or EC_{50})	PPARγ (IC_{50} or EC_{50})	PPARδ (IC_{50} or EC_{50})	Activity	Sizes
AM3102 Catalog No. 13452			0.1 µM	Agonist	5 mg • 10 mg • 25 mg • 50 mg
BADGE Catalog No. 70790	100 µM (K_d)			Antagonist	25 g • 50 g • 100 g • 500 g
Bezafibrate Catalog No. 10009145	60 µM (human)	20 µM (human)	50 µM (human)	Agonist; Lowers LDL & Triglycerides; Raises HDL	500 mg • 1 g • 5 g • 10 g
CAY10506 Catalog No. 10009079	10 µM			Agonist	1 mg • 5 mg • 10 mg • 50 mg
CAY10514 Catalog No. 10009017	0.64 µM		0.17 µM	Dual Agonist	1 mg • 5 mg • 10 mg • 50 mg
CAY10573 Catalog No. 10008846	0.05 µM	0.223 µM	0.113 µM	Agonist	500 µg • 1 mg • 5 mg • 10 mg
CAY10592 Catalog No. 10012536		0.030-0.053 µM		Agonist	1 mg • 5 mg • 10 mg • 50 mg
CAY10599 Catalog No. 13282	0.050 µM	>10 µM	4 µM	Agonist	1 mg • 5 mg • 10 mg • 25 mg
Ciglitazone Catalog No. 71730	3 µM			Agonist; Antidiabetic Drug	1 mg • 5 mg • 10 mg • 50 mg
Clofibrate Catalog No. 10005745			55 µM (human)	Agonist; Treat Dyslipidemia	500 ml • 1 ml • 5 ml • 10 ml
Fenofibrate Catalog No. 10005368			30 µM (human)	Agonist; Treat Dyslipidemia	1 g • 5 g • 10 g • 50 g
GW 0742 Catalog No. 10006798		0.0011 µM (human)		Agonist	5 mg • 10 mg • 25 mg • 50 mg
GW 7647 Catalog No. 10008613	1.1 µM (human)	6.2 µM (human)	0.006 µM (human)	Agonist	1 mg • 5 mg • 10 mg • 25 mg
GW 9578 Catalog No. 10011211			0.05 µM (human)	Agonist	500 µg • 1 mg • 5 mg • 10 mg
GW 9662 Catalog No. 70785	>90% inhibition at 0.1 µM			Antagonist	1 mg • 5 mg • 10 mg • 50 mg
GW 590735 Catalog No. 10009880			0.004 µM	Agonist	1 mg • 5 mg • 10 mg • 25 mg
N-Octadecyl-N′-propyl-sulfamide Catalog No. 10009661			0.1 µM	Agonist	5 mg • 10 mg • 25 mg • 50 mg
Oleoyl Ethanolamide Catalog No. 90265			0.12 µM	Agonist	5 mg • 10 mg • 50 mg • 100 mg
15-deoxy-Δ^{12,14}-Prostaglandin J_2 Catalog No. 18570	2 µM			Agonist	100 µg • 500 µg • 1 mg • 5 mg
Rosiglitazone Catalog No. 71740	0.043 µM (K_d)			Agonist	5 mg • 10 mg • 50 mg • 100 mg
T0070907 Catalog No. 10026	0.001 µM			Antagonist	1 mg • 5 mg • 10 mg • 50 mg

Also Available: 15-deoxy-Δ^{12,14}-Prostaglandin J_2-biotin (10141) • Rosiglitazone (potassium salt) (71742) • 15-deoxy-Δ^{12,14}-Prostaglandin J_2 (18570.1) • 15-deoxy-Δ^{12,14}-Prostaglandin J_2-d_4 (318570) • 15-deoxy-Δ^{12,14}-Prostaglandin J_2 Quant-PAK (10006850) • 15-deoxy-Δ^{12,14}-Prostaglandin J_2 Lipid Maps MS Standard (10007235) • Rosiglitazone-d_3 Maleate (10011343)

PPARδ (human recombinant) 10007451

FAAR, NUC1, Nuclear Hormone Receptor 1, PPARβ
M_r: 54 kDa **Purity:** ≥95%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 20% glycerol, 150 mM sodium chloride, and 1 mM DTT
Source: Recombinant protein isolated from a baculovirus overexpression system in *Sf*21 cells

10 µg
25 µg
50 µg

•Also Available: **PPARδ Western Ready Control** (10009568)

PPARγ FL (human recombinant from *E. coli*) 61700

PPARγ Full Length
M_r: ~60 kDa **Purity:** ≥90% by SDS-PAGE
Supplied in: 20 mM Tris HCl, pH 8.0, containing 250 mM KCl, 20% glycerol, 5 mM DTT, and 0.5 mM EDTA
Source: Recombinant N-terminal His-tagged protein expressed in *E. coli*

5 µg
10 µg
25 µg
50 µg

PPARγ FL (human recombinant from *Sf*21 cells) 10009987

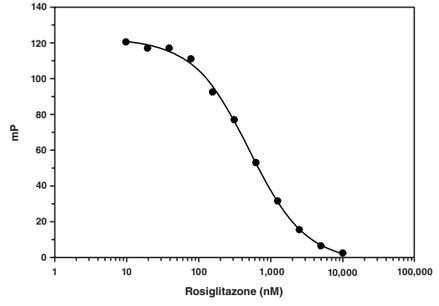
PPARγ Full Length
M_r: ~60 kDa **Purity:** ≥80% by SDS-PAGE
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride, 20% glycerol, 1 mM DTT, and 20% mM glycerol
Source: Recombinant N-terminal His-tagged protein expressed in *Sf*21 cells

5 µg
10 µg
25 µg
50 µg

PPARγ FP-Based Ligand Screening Assay Kit - Green 10007685

Stability: ≥6 months at -20°C
Summary: Cayman's PPARγ FP-Based Ligand Screening Assay - Green provides a convenient fluorescence polarization (FP)-based single step assay for screening PPARγ ligands. In this assay, a ligand of PPARγ was conjugated to FITC and is used as the displacement probe. Agonists and antagonists of PPARγ will displace the fluorescent probe leading to a decrease in FP. The PPARγ FP-Based Ligand Screening Assay is a robust assay with a Z' of 0.81 and has a dynamic range of greater than 120 mP units. The assay has been validated using known agonists/ligands of PPARγ (Arachidonic Acid, Rosiglitazone, Troglitazone, etc.) with IC_{50} values ranging from nanomolar to millimolar concentrations.

384 wells
1,920 wells



PPARγ LBD (human recombinant) 10007941

PPARγ Ligand Binding Domain
M_r: ~34 kDa **Purity:** ≥90%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 20% glycerol, 150 mM sodium chloride, and 1 mM DTT
Source: Recombinant N-terminal His-tagged protein expressed in *E. coli*

25 µg
50 µg
100 µg

PPARγ-PAK 71000

Purity: ≥98% **Stability:** ≥1 year at -20°C
Summary: Contains ciglitazone, GW 9662, 15-deoxy-Δ^{12,14}-PGJ_2, rosiglitazone, and troglitazone

1 ea

PRIMA-1 63520

[5608-24-2]
MF: $C_9H_{15}NO_3$ **FW:** 185.2 **Purity:** ≥95%
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: A unique anti-oncogenic substance that acts as a re-activator of the apoptotic function of mutant p53

1 mg
5 mg
10 mg
50 mg

2,2-bis(hydroxymethyl)-3-quinuclidinone

PRMT4 Polyclonal Antibody 13552

CARM1
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human PRMT4 amino acid sequences 45-69 and 595-608 • Host: rabbit • Cross Reactivity: (+) human PRMT4 • Application(s): WB

1 ea

PRMT5 Polyclonal Antibody 13559

JBP1, Skb1 HS
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: synthetic peptide from human PRMT5 • Host: rabbit • Cross Reactivity: (+) human PRMT5 • Application(s): WB

1 ea

PRMT6 Polyclonal Antibody 13558

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human PRMT6 amino acids 23-43 • Host: rabbit • Cross Reactivity: (+) human and murine PRMT6 • Application(s): WB

1 ea

PRMT7 Polyclonal Antibody 13551

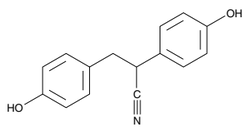
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human PRMT7 amino acids 346-360 • Host: rabbit • Cross Reactivity: (+) human and murine PRMT7 • Application(s): WB

1 ea

2,3-*bis*(4-hydroxyphenyl) Propionitrile 10008842

[1428-67-7] DPN
MF: $C_{15}H_{13}NO_2$ **FW:** 239.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An ERα selective agonist; exhibits 70-fold higher relative binding affinity for ERα *versus* ERβ

5 mg
10 mg
50 mg
100 mg

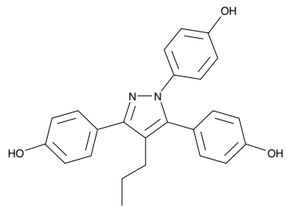


4-hydroxy-α-(4-hydroxyphenyl)-benzenepropanenitrile

Propylpyrazole Triol 10008841

[263717-53-9] PPT
MF: $C_{24}H_{22}N_2O_3$ **FW:** 386.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An ERα selective agonist with a 410-fold relative binding affinity for ERα *versus* ERβ

1 mg
5 mg
10 mg
25 mg

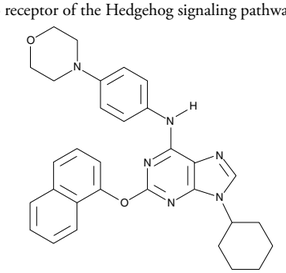


4,4',4"-(4-propyl-1H-pyrazole-1,3,5-triyl)tris-phenol

Purmorphamine 10009634

[483367-10-8]
MF: $C_{31}H_{32}N_6O_2$ **FW:** 520.6 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A 2,6,9-trisubstituted purine that promotes the differentiation of both human and murine mesenchymal progenitor cells into osteoblasts; binds to and activates the 7-transmembrane Smo receptor of the Hedgehog signaling pathway

1 mg
5 mg
10 mg
25 mg

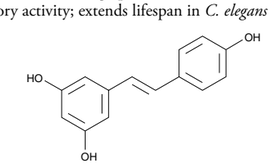


9-cyclohexyl-N-[4-(morpholinyl)phenyl]-2-(1-naphthalenyloxy)-9H-purin-6-amine

trans-Resveratrol 70675

[501-36-0] (E)-Resveratrol
MF: $C_{14}H_{12}O_3$ **FW:** 228.2 **Purity:** ≥98%
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: A potent phenolic antioxidant found in grapes and red wine that also has antiproliferative and anti-inflammatory activity; extends lifespan in *C. elegans*

50 mg
100 mg
250 mg
500 mg



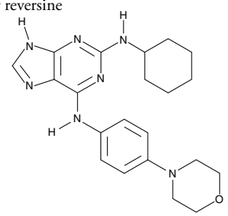
5-[(1E)-2-(4-hydroxyphenyl)ethenyl]-1,3-benzenediol

- Also Available: ***cis*-Resveratrol** (10004235)
  ***cis*-Resveratrol-d_4** (13128)
  ***cis*-trismethoxy Resveratrol** (13199)
  ***trans*-Resveratrol-d_4** (13130)
  ***trans*-trismethoxy Resveratrol** (10188)
  ***trans*-trismethoxy Resveratrol-d_4** (13129)

Retreversine 10006606

MF: $C_{21}H_{27}N_7O$ **FW:** 393.5 **Purity:** ≥98%
A solution in ethanol **Stability:** ≥2 years at -20°C
Summary: An inactive control analog of reversine

500 µg
1 mg
5 mg
10 mg

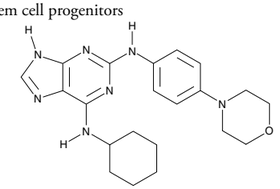


N2-cyclohexyl-N6-[4-(4-morpholinyl)phenyl]-1H-purine-2,6-diamine

Reversine 10004412

[656820-32-5]
MF: $C_{21}H_{27}N_7O$ **FW:** 393.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A 2,6-disubstituted purine derivative that causes dedifferentiation of cultured myoblasts into confluent stem cell progenitors

1 mg
5 mg
10 mg
25 mg

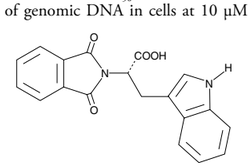


N6-cyclohexyl-N2-[4-(4-morpholinyl)phenyl]-1H-purine-2,6-diamine

RG-108 13302

[48208-26-0] N-Phthalyl-L-Tryptophan
MF: $C_{19}H_{14}N_2O_4$ **FW:** 334.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A non-nucleoside DNA MT inhibitor (IC_{50} = 115 nM *in vitro*) that significantly reduces the methylation of genomic DNA in cells at 10 µM without detectable toxicity

5 mg
10 mg
50 mg
100 mg

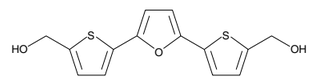


α-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-(αS)-1H-indole-3-propanoic acid

RITA 10006426

[213261-59-7] 2,5-bis(5-hydroxymethyl-2-thienyl) Furan, NSC 652287
MF: $C_{14}H_{12}O_3S_2$ **FW:** 292.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of p53-HDM-2 interaction that can reactivate the tumor suppressor function of wild-type p53; binds to p53 with an apparent K_d value of 1.5 nM and prevents interaction with HDM-2 resulting in p53 stabilization, accumulation and activation

1 mg
5 mg
10 mg
50 mg

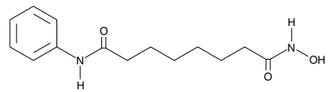


5,5'-(2,5-furandiyl)bis-2-thiophenemethanol

SAHA 10009929

[149647-78-9] Suberoylanilide Hydroxamic Acid, Vorinostat, Zolinza™
MF: $C_{14}H_{20}N_2O_3$ **FW:** 264.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A HDAC inhibitor of class I and class II HDACs at around 50 nM; arrests cell growth in a wide variety of transformed cells in culture at 2.5-5.0 µM

50 mg
100 mg
250 mg
500 mg

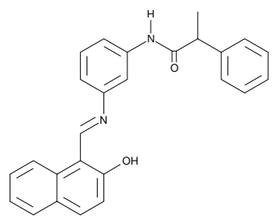


N1-hydroxy-N8-phenyl-octanediamide

Salermide 13178

[1105698-15-4]
MF: $C_{26}H_{22}N_2O_2$ **FW:** 394.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An inhibitor of SIRT1 and SIRT2, causing tumor-specific apoptotic cell death; causes 90% apoptosis within 72 hours (IC_{50} = ~20 µM) by reactivating proapototic genes that are repressed by SIRT1 in MOLT4 leukemia cells

5 mg
10 mg
50 mg
100 mg

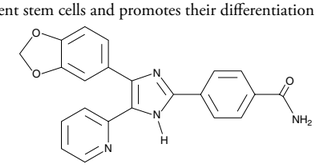


N-[3-[[(2-hydroxy-1-naphthalenyl)methylene]amino]phenyl]-α-methyl-benzeneacetamide

SB 431542 13031

[301836-41-9]
MF: $C_{22}H_{18}N_4O_3$ **FW:** 384.4 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent and selective inhibitor of the TGF-β1 receptors ALK5 (IC_{50} = 94 nM), ALK4 (IC_{50} = 140 nM) and, less effectively, ALK7; suppresses renewal in embryonic and induced pluipotent stem cells and promotes their differentiation

1 mg
5 mg
10 mg
25 mg

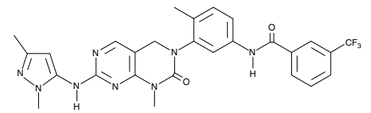


4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide

SC-1 10009557

Pluripotin
MF: $C_{27}H_{25}F_3N_8O_2$ **FW:** 550.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A small molecule activator of stem cell renewal that allows the propagation of OG2 mES cells for at least 10 passages in an undifferentiated state; activity is mediated by the combined inhibition of RasGAP and ERK1 with K_d values of 98 and 212 nM, respectively

1 mg
5 mg
10 mg
25 mg

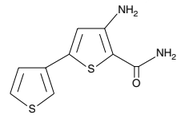


N-(3-(7-(1,3-dimethyl-1H-pyrazol-5-ylamino)-1-methyl-2-oxo-1,2-dihydropyrimido[4,5-d]pyrimidin-3(4H)-yl)-4-methylphenyl)-3-(trifluoromethyl) benzamide

SC-514 10010267

[354812-17-2]
MF: $C_9H_8N_2OS_2$ **FW:** 224.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A selective and reversible inhibitor of IKK2 (IC_{50} = 3-12 µM) that displays >10-fold selectivity over 28 other kinases; attenuates NF-κB-mediated gene expression in synovial fibroblasts, smooth muscle cells, and astrocytes

5 mg
10 mg
25 mg
50 mg



4-amino-[2,3'-bithiophene]-5-carboxamide

SET7/9 (human recombinant) 10320

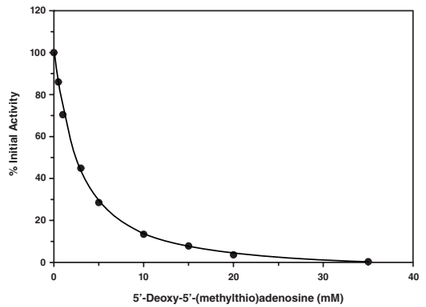
KMT7, SETD7/9, SET Domain-Containing Protein 7/9
M_r: 43.3 kDa **Purity:** ≥95%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal His-tagged full-length enzyme (amino acids 1-366) expressed in *E. coli*

25 µg
50 µg
100 µg

NEW SET7/9 Methyltransferase Inhibitor Screening Assay Kit 700270

KMT7, SETD7/9, SET Domain-Containing Protein 7/9
Stability: ≥6 months at -80°C
Summary: SET7/9 is a MT that acts on various substrates including histone 3 at lysine residue 4 (H3K4), p53, and the transcription factor TAF 10. Unlike most SET proteins, SET7/9 is exclusively a mono-methylase. Cayman's SET7/9 MT Inhibitor Screening Assay provides a convenient method for screening SET7/9 inhibitors. The transfer of the methyl group from SAM by SET7/9 to the acceptor peptide (TAF 10) generates SAH, which is rapidly converted to urate and H_2O_2 using an enzyme mixture provided in the kit. A subsequent reaction between H_2O_2 and ADHP (10-acetyl-3,7-dihydroxyphenoxazine) produces the highly fluorescent compound resorufin. Resorufin fluorescence is analyzed using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

96 wells



SET7/9 Polyclonal Antibody 13731

KMT7, SETD7/9, SET Domain-Containing Protein 7/9
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human SET7/9 amino acids 131-145 and 336-352 • Host: rabbit • Cross Reactivity: (+) murine and human SET7/9 • Application(s): WB

1 ea

NEW SET7/9(FL) Polyclonal Antibody 13780

KMT7, SETD7/9, SET Domain-Containing Protein 7/9
Supplied as: Protein A-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human SET7/9 amino acids 1-366 • Host: rabbit • Cross Reactivity: (+) murine and human SET7/9 • Application(s): WB

1 ea

NEW SET8 (human recombinant) 10319

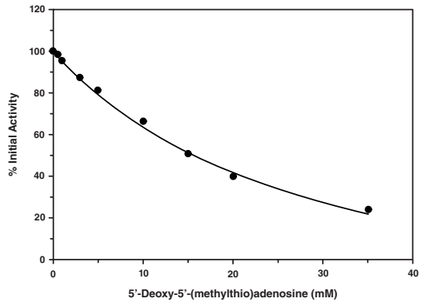
PR-Set7, SETD8, SET Domain-Containing (lysine methyltransferase) 8
M_r: 21.1 kDa **Purity:** ≥95%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal His-tagged protein (amino acids 190-352) expressed in *E. coli*

25 µg
50 µg
100 µg

NEW SET8 Methyltransferase Inhibitor Screening Assay Kit 700350

KMTSA, PR-Set7, SETD8, SET Domain-Containing (lysine methyltransferase) 8
Stability: ≥6 months at -80°C
Summary: SET Domain-containing Protein 8 (SET8) is a MT that selectively mono-methylates histone H4 at lysine residue 20 (H4K20), an event proven to have an important role in chromatin structure and transcriptional activation. Cayman's SET8 MT Inhibitor Screening Assay provides a convenient method for screening human SET8 inhibitors. The transfer of the methyl group from SAM by SET8 (provided in the kit) to the acceptor peptide generates S-adenosylhomocysteine, which is rapidly converted to urate and H_2O_2 using an enzyme mixture provided in the kit. The H_2O_2 formed reacts with ADHP (10-acetyl-3,7-dihydroxyphenoxazine) to produce the highly fluorescent compound resorufin (excitation 530-540 nm; emission 585-595 nm).

96 wells



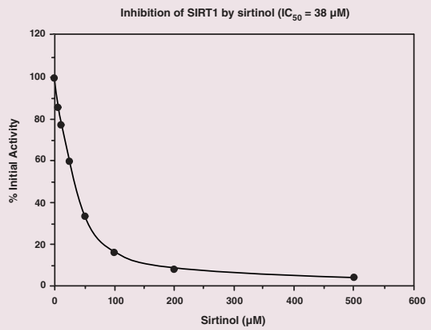
SIRT Direct Fluorescent Screening Assay Kits

The sirtuins represent a distinct class of trichostatin A-insensitive lysyl-deacetylases (class III HDACs) that catalyze a reaction coupling lysine deacetylation to the formation of nicotinamide and O-acetyl-ADP-ribose. Cayman's Direct Fluorescent Screening Assay Kits provide a convenient fluorescence-based method for screening SIRT inhibitors or activators. The procedure requires only two easy steps, both performed in the same microplate. In the first step, the substrate is incubated with human recombinant SIRT along with its cosubstrate NAD^+. Deacetylation sensitizes the substrate such that treatment with the developer in the second step releases a fluorescent product. The fluorophore can be analyzed with an excitation wavelength of 350-360 nm and an emission wavelength of 450-465 nm.

SIRT1 Direct Fluorescent Screening Assay Kit 10010401

Stability: ≥6 months at -80°C

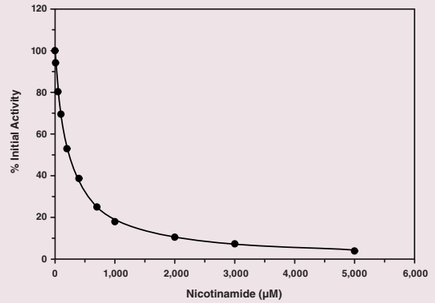
96 wells



NEW SIRT2 Direct Fluorescent Screening Assay Kit 700280

Stability: ≥6 months at -80°C

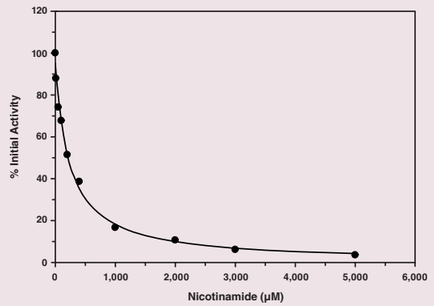
96 wells



SIRT3 Direct Fluorescent Screening Assay Kit 10011566

Stability: ≥6 months at -80°C

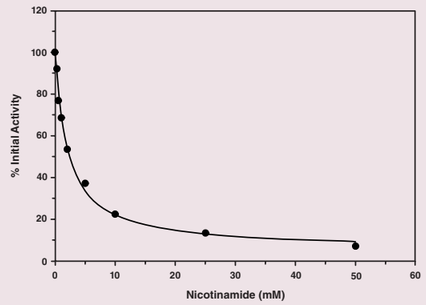
96 wells



NEW SIRT6 Direct Fluorescent Screening Assay Kit 700290

Stability: ≥6 months at -80°C

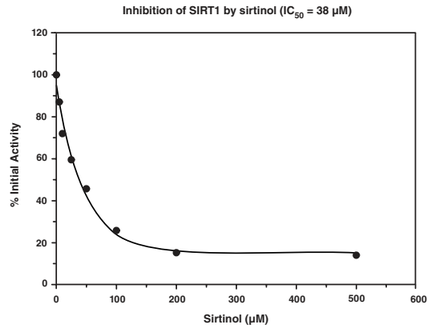
96 wells



SIRT1 FRET-Based Screening Assay Kit 10010991

Stability: ≥6 months at -80°C
Summary: Human SIRT1 is the homolog of yeast Sir2 and has been shown to regulate the activity of the p53 tumor suppressor and inhibit apoptosis. Cayman's SIRT1 FRET-Based Screening Assay provides a convenient fluorescence-based method for screening SIRT1 inhibitors or activators that differs from the other fluorescence-based methods of measuring SIRT activity. The procedure requires only two easy steps, both performed in the same microplate. In the first step, the substrate, which is coupled to the fluorophore and quencher, is incubated with human recombinant SIRT1 along with its cosubstrate NAD^+. Deacetylation sensitizes the substrate such that treatment with the developer in the second step results in the separation of the quencher and fluorophore. The resulting fluorescence is analyzed using an excitation wavelength of 335-345 nm and emission wavelength of 440-465 nm.

96 wells



SIRT1 (human recombinant) 10011190

Sirtuin 1, SIR2, SIR2L1
M_r: 89.2 kDa **Purity:** ≥60%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal GST-tagged SIRT1 amino acids 193-747 expressed in *E. coli*

25 Units
50 Units
100 Units

SIRT2 (human recombinant) 10011191

SIR2L
M_r: 44.2 kDa **Purity:** ≥90%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal His-tagged SIRT2 amino acids 2-389 expressed in *E. coli*

25 µg
50 µg
100 µg

SIRT3 (human recombinant) 10011194

SIR2L3
M_r: 37.0 kDa **Purity:** ≥90%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal His-tagged SIRT3 amino acids 101-399 expressed in *E. coli*

25 µg
50 µg
100 µg

NEW SIRT4 (human recombinant) 10317

SIR2L4
M_r: 61.9 kDa **Purity:** ≥85%
Supplied in: 50 mM sodium phosphate, pH 7.2, 100 mM sodium chloride, 2.5 mM DTT, and 20% glycerol
Source: Recombinant N-terminal GST-tagged SIRT4 expressed in *E. coli*

25 µg
50 µg
100 µg

SIRT5 (human recombinant) 10318

SIR2L5
M_r: 60.6 kDa **Purity:** ≥90%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal GST-tagged SIRT5 expressed in *E. coli*

25 µg
50 µg
100 µg

SIRT6 (human recombinant) 10315

SIR2L6
M_r: 43.7 kDa **Purity:** ≥95%
Supplied in: 25 mM Tris, pH 8.0, containing 100 mM sodium chloride and 20% glycerol
Source: Recombinant N-terminal His-tagged SIRT6 (amino acids 1-355) expressed in *E. coli*

25 µg
50 µg
100 µg

NEW SIRT7 (human recombinant) 10316

SIR2L7
M_r: 49.3 kDa **Purity:** ≥85%
Supplied in: 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride, 5 mM DTT, and 20% glycerol
Source: Recombinant N-terminal His-tagged SIRT7 expressed in *E. coli*

25 µg
50 µg
100 µg

SIRT7 Polyclonal Antibody 13477

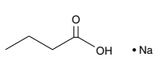
SIR2L7
Supplied as: Affinity-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human SIRT7 amino acids 35-51 and 361-377 • Host: rabbit • Cross Reactivity: (+) human SIRT7 • Application(s): WB

1 ea

Sodium Butyrate 13121

[156-54-7] Butyric Acid (sodium salt)
MF: $C_4H_8O_2$ • Na **FW:** 111.1 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at Room Temperature
Summary: A short chain fatty acid that inhibits HDACs, induces growth arrest, differentiation and apoptosis in cancer cells, and suppresses inflammation by reducing the expression of pro-inflammatory cytokines

50 g
100 g
250 g
500 g

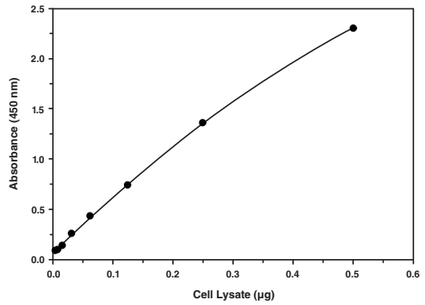


butanoic acid, sodium salt

SREBP-1 Transcription Factor Assay Kit 10010854

Stability: ≥1 year at -80°C
Summary: Three known isoforms of SREBP transcription factors have been characterized: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1c acts primarily to activate genes required for fatty acid synthesis, such as acetyl CoA carboxylase, fatty acid synthase, and long chain fatty acid elongase. In addition, SREBP-1c may also contribute to the regulation of glucose uptake and synthesis through induction of glucokinase. SREBP-1c has important clinical implications in the treatment of many diseases including obesity, diabetes mellitus, insulin resistance, and non-alcoholic fatty liver disease. Cayman's SREBP-1 Transcription Factor Assay is a sensitive colorimetric method for detecting SREBP-1 transcription factor binding activity from nuclear exctracts and whole cell lysates in a 96-well format.

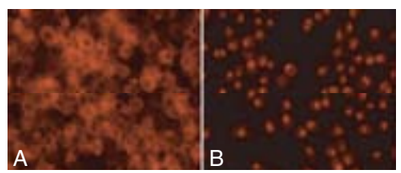
96 wells



SREBP-2 Cell-Based Translocation Assay Kit 10009239

Stability: ≥1 year at -20°C
Summary: SREBP-2 is a transcription factor that regulates cholesterol synthesis by activating the expression of genes for HMG-CoA reductase and other enzymes of the cholesterol synthetic pathway. Cayman's SREBP-2 Cell-Based Translocation Assay Kit provides the tools needed to study SREBP-2 movement within whole cells. The kit contains a highly specific SREBP-2 primary antibody together with a DyLight™ (trademarked by Thermo Fisher Scientific) conjugated secondary antibody in a ready to use format. Also included as a positive control is a cholesterol trafficking inhibitor, U18666A, which has been shown to activate SREBP-2 translocation into nuclei by scientists at Cayman Chemical Company.

96 wells



Translocation of SREBP-2 into nuclei by 24 µM U-18666A. Raw 264.7 cells were seeded in a 96-well plate at a density of 3 x 10^4 cells/well and cultured overnight. The next day, cells were treated with DMSO (vehicle) or 24 µM U-18666A for 72 hours. *Panel A*: Cells treated with DMSO alone demonstrate cytoplasmic localization of SREBP-2, indicating that most of cells have inactive protein. *Panel B*: U-18666A treatment for three days induced SREBP-2 translocation into the nuclei, indicating that blockage of cholesterol transport in these cells activates the protein.

SREBP-2 Polyclonal Antibody 10007663

SREBF-2
Supplied as: Peptide affinity-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: human SREBP-2 amino acids 455-469 • Host: rabbit • Cross Reactivity: (+) human, murine, and rat SREBP-2 • Application(s): ICC and WB

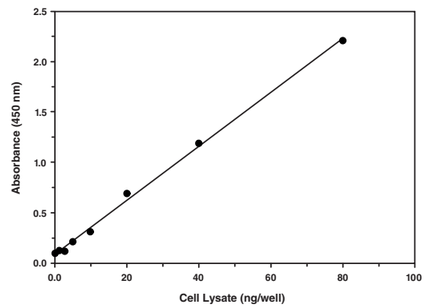
1 ea

• Also Available: **SREBP-2 Blocking Peptide** (10009266)

SREBP-2 Transcription Factor Assay Kit 10007819

Stability: ≥6 months at -20°C
Summary: SREBP-2 is a transcription factor that performs a critical role in the transcriptional regulation of genes involved in cholesterol synthesis and uptake including HMG-CoA synthase, HMG-CoA reductase, and the LDL receptor. Cayman's SREBP-2 Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates in a 96-well format. A specific dsDNA sequence containing the SREBP response element is immobilized to the wells of a 96-well plate. SREBP contained in a nuclear extract, binds specifically to the SREBP response element. SREBP is detected by addition of specific primary antibody directed against SREBP. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

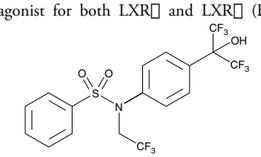
96 wells



T0901317 71810

[293754-55-9]
MF: $C_{17}H_{12}F_9NO_3S$ **FW:** 481.3 **Purity:** ≥98%
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: A potent and selective agonist for both LXR and LXR (EC_{50} = 50 nM)

5 mg
10 mg
50 mg
100 mg



N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl] phenyl]-benzenesulfonamide

TAF10 Peptide 10228

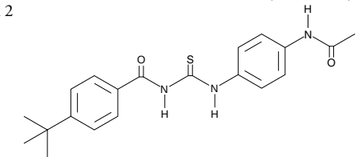
TBP-Associated Factor 10, TAFII30, TAF10 RNA polymerase II, TATA Box Binding Protein (TBP)-Associated Factor
FW: 1,267.0 **Supplied as:** 1 mg peptide lyophilized peptide from ammonium bicarbonate buffer **Stability:** ≥1 year at -20°C
Summary: TAF10 is one of many protein factors or coactivators associated with RNA polymerase II activity. One vial of this peptide may be used as a MT acceptor peptide for more than 200 reactions at 15 µM

1 ea

Tenovin-1 13085

[380315-80-0]
MF: $C_{20}H_{23}N_3O_2S$ **FW:** 369.5 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A small molecule activator of p53 that decreases the growth of BL2 Burkitt's lymphoma and ARN8 melanoma cells; inhibits the deacetylase activity of purified human SIRT1 and 2

5 mg
10 mg
50 mg
100 mg

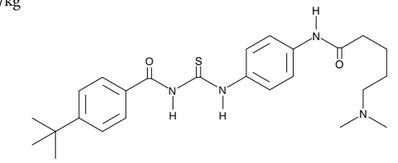


N-[[[4-(acetylamino)phenyl]amino]thioxomethyl-4-(1,1-dimethylethyl)]-benzamide

Tenovin-6 13086

[1011557-82-6]
MF: $C_{25}H_{34}N_4O_2S$ **FW:** 454.6 **Purity:** ≥95%
A crystalline solid **Stability:** ≥1 year at -20°C
Summary: A water-soluble analog of tenovin-1; elevates p53 expression in MCF-7 cells at 10 µM and reduces growth of ARN8 melanoma xenograft tumors in SCID mice at a dose of 50 mg/kg

1 mg
5 mg
10 mg
25 mg

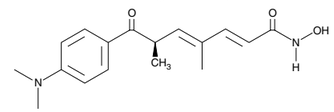


N-[[[4-[[5-(dimethylamino)-1-oxopentyl]amino]phenyl]amino]thioxomethyl]-4-(1,1-dimethylethyl)-benzamide

Trichostatin A 89730

[58880-19-6] TSA
MF: $C_{17}H_{22}N_2O_3$ **FW:** 302.4 **Purity:** ≥98%
A solution in ethanol **Stability:** ≥1 year at -20°C
Summary: A potent, reversible inhibitor of HDAC (IC_{50} = 70 nM)

500 µg
1 mg
5 mg
10 mg

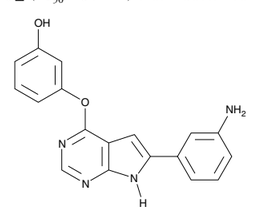


7-[4-(dimethylamino)phenyl]-N-hydroxy-4,6R-dimethyl-7-oxo-2E,4E-heptadienamide

TWS119 10011251

[601514-19-6]
MF: $C_{18}H_{14}N_4O_2$ **FW:** 318.3 **Purity:** ≥90%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A potent inhibitor of GSK3 (IC_{50} = 30 nM) that induces neurogenesis in murine embryonic stem cells

1 mg
5 mg
10 mg
25 mg



3-[[6-(3-aminophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]oxy]-phenol

NEW Ubiquitin Monoclonal Antibody (Clone 5B9-B3) 13722

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: native bovine ubiquitin conjugated to KLH • Host: mouse, clone 5B9-B3 • Isotype: IgG_{2ak} • Cross Reactivity: (+) human, murine, rat, and bovine ubiquitin • Application(s): ELISA and WB

50 µg
200 µg

NEW Ubiquitin Monoclonal Antibody (Clone 6C11-B3) 13723

Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: native bovine ubiquitin conjugated to KLH • Host: mouse, clone 6C11-B3 • Isotype: IgG_{2ak} • Cross Reactivity: (+) human, murine, rat, and bovine ubiquitin • Application(s): ELISA and WB

50 µg
200 µg

NEW Ubiquitin Polyclonal Antibody 13724

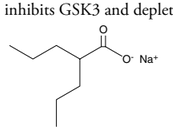
Supplied as: Protein G-purified IgG **Stability:** ≥1 year at -20°C
Summary: Antigen: native bovine ubiquitin conjugated to KLH • Host: rabbit • Cross Reactivity: (+) human, monkey, murine, rat, hamster, rabbit, guinea pig, bovine, porcine, canine, ovine, chicken, *Xenopus*, yeast, *Drosophila*, and rainbow trout ubiquitin • Application(s): ChIP, IP, and WB

50 µg
200 µg

Valproic Acid (sodium salt) 13033

[1069-66-5] 2-Propylvaleric Acid, Sodium Valproate
MF: $C_8H_{16}O_2$ • Na **FW:** 167.2 **Purity:** ≥95%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: An analog of valeric acid, long used as an anti-convulsant; inhibits Class I HDACs with an IC_{50} value of ~2 mM; also inhibits GSK3 and depletes cellular IP_3

10 g
25 g
50 g
100 g

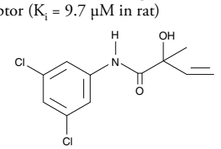


2-propyl-pentanoic acid, monosodium salt

Vinclozolin M2 10007452

[83792-61-4] M2
MF: $C_{11}H_{11}Cl_2NO_2$ **FW:** 260.1 **Purity:** ≥98%
A crystalline solid **Stability:** ≥2 years at -20°C
Summary: A metabolite of vinclozolin, a dicarboximide fungicide, that acts as an effective antagonist of the androgen receptor (K_i = 9.7 µM in rat)

5 mg
10 mg
25 mg
50 mg



N-(3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenamide

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