

Luciferase Assay Kits

Firefly and Renilla luciferase are widely used as transcriptional reporter genes for research and drug screening. Biotium offers both high sensitivity flash type and steady-glow type luciferase assay systems for single or dual luciferase assays for research and high throughput screening (HTS). ATP-Glo™ is a highly sensitive assay kit for quantitating live cell numbers or ATP levels based on firefly luciferase luminescence. Biotium's new luciferase assay kits in lyophilized format can be shipped at room temperature to save you money and increase product reliability.

Flash Type Luciferase Reporter Assays

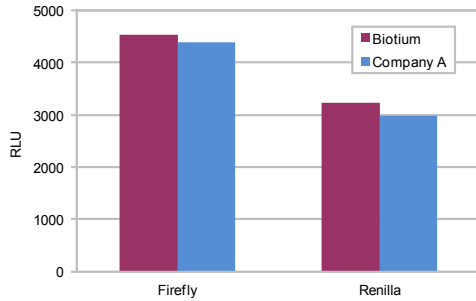


Figure 1. Luciferase activity (relative luminescence units; RLU) was assayed using PC3 cells transfected with firefly and Renilla luciferase expression vectors using Biotium's luciferase assay kits or kits from a leading competitor.

Steady-Luc™ Glow Type Luciferase Reporter Assay

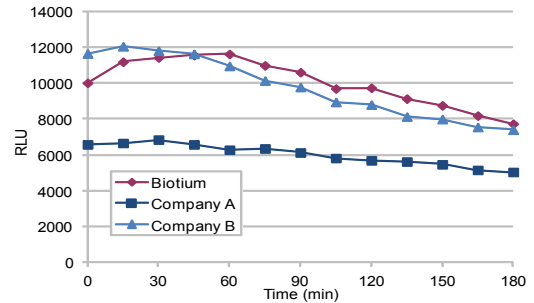


Figure 2. Luciferase activity was assayed using PC3 cells transfected with firefly luciferase expression vector. Luminescence signal (relative luminescence units; RLU) was measured every 15 minutes for 3 hours.

Features:

- Flash-type assay for single luminescence readings
- High sensitivity & broad linear range
- Low autoluminescence
- Lyophilized firefly kit available for convenient shipping and storage

Features:

- Glow-type assay for single tube or multi-well format
- One-step, homogenous HTS assay
- High sensitivity & broad linear range
- Lyophilized format available for convenient shipping and storage

ATP-Glo™ Bioluminescent Cell Viability Assay

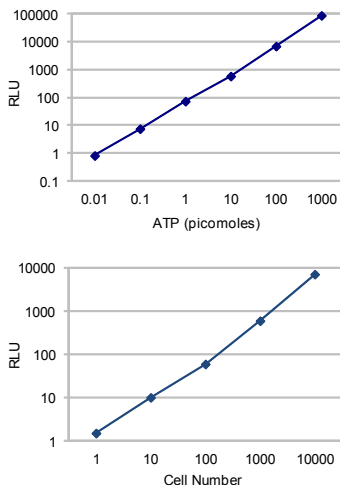


Figure 3. Measurement of ATP (top) or Jurkat cells (bottom) using ATP-Glo Bioluminescent Cell Viability Assay Kit.

Features:

- High sensitivity & broad linearity
- One step, homogenous assay
- Quantitate ATP or viable cells
- For single tube luminometer or luminescence plate reader with injector

Product	Cat. No.	Unit Size
Firefly Luciferase Assay Kit	30003-T	50 assays
	30003-1	150 assays
	30003-2	1000 assays
NEW! Firefly Luciferase Assay Kit (Lyophilized)	30075-1	150 assays
	30075-2	1000 assays
Renilla Luciferase Assay Kit	30004-T	50 assays
	30004-1	150 assays
	30004-2	1000 assays
Firefly & Renilla Dual Luciferase Assay Kit	30005-T	50 assays
	30005-1	100 assays
	30005-2	1000 assays
Steady-Luc™ Firefly HTS Assay Kit	30028-T	40 assays
	30028-1	100 assays
	30028-2	1000 assays
	30028-3	10,000 assays
NEW! Steady-Luc™ Firefly HTS Assay Kit (Lyophilized)	30028-L1	100 assays
	30028-L2	1000 assays
	30028-L3	10,000 assays
ATP-GLO™ Bioluminescent Cell Viability Assay	30020-T	50 assays
	30020-1	200 assays
	30020-2	1000 assays

Luminescence Substrates

Luminescent enzymes are commonly used reporter genes *in vitro*, and recently have proved to be highly sensitive tools for small animal imaging as well, due to the absence of endogenous luciferase activity in mammals, and because cells and tissue exhibit very low autoluminescence. Biotium offers high purity D-luciferin and coelenterazine luminescent substrates.

D-Luciferin is a substrate for the widely-used reporter enzyme firefly luciferase. The enzyme catalyzes ATP-dependent D-luciferin oxidation to oxyluciferin, producing light emission centered at 560 nm.

Coelenterazine is a substrate for Renilla (sea pansy) luciferase, as well as other enzymes such as *Gaussia*, *Metridia*, and *Oplophorus* luciferases. Renilla luciferase catalyzes coelenterazine oxidation by oxygen to produce light, and is a widely used reporter gene for luminescence based assays. Coelenterazine native is the natural substrate for Renilla luciferase. In addition, over a dozen of coelenterazine analogs have been synthesized that can function as substrates for Renilla luciferase, and have different luminescent properties, summarized in Table 1. In addition to quantum yields, emission wavelength can be an important factor when luciferase is used in combination with a fluorescent protein such as GFP for bioluminescent resonance energy transfer (BRET), an important application for the studies of protein-protein interactions.

Coelenterazine and its analogs also bind the jellyfish protein apoaequorin to form aequorin, a calcium-sensing photoprotein. Aequorin can be used for bioluminescent detection of calcium with high sensitivity and dynamics. Compared with fluorescent calcium indicators, aequorin has several advantages in detecting calcium. One major advantage is that the aequorin complex can detect a broad range of calcium concentrations, from ~0.1 μM to >100 μM . Another advantage is that the aequorin complex is stably retained inside cells, making it possible to follow calcium concentration changes for hours to days. Table 2 lists the luminescent properties of coelenterazine analogs in complex with apoaequorin.

Coelenterazines are poorly water soluble, which complicates the formulation of these substrates for use in live animals. Aquaphile™ coelenterazines are specially formulated to readily dissolve in water or buffer for *in vivo* dosing.

Table 1 Luminescent Properties of Coelenterazine Analogs with Renilla Luciferase*

Analog	λ_{em} (nm)	Total Light (%)	Initial Intensity (%)
native	475	100	45
400a	400		
cp	470	23	135
e	418, 475	137	900
f	473	28	45
h	475	41	135
n	475	47	900

* Data from Inouye, S and Shimomura, O. (1997). *Biochem. Biophys. Res. Commun.* 233, 349-353.

Table 2. Luminescent Properties of Coelenterazine Analogs with Apoaequorin*

Analog	λ_{em} (nm)	Relative luminescence capacity	Relative intensity	Half-rise time (s)
native	465	1.0	1.00	0.4-0.8
cp	442	0.95	15	0.15-0.3
e	405, 465	0.50	4	0.15-0.3
f	473	0.80	18	0.4-0.8
fcp	452	0.57	135	0.4-0.8
h	475	0.82	10	0.4-0.8
hcp	444	0.67	190	0.15-0.3
i	476	0.70	0.03	8
ip	441	0.54	47	1
n	467	0.26	0.01	5

* Data from Shimomura, B, et al. (1989). *Biochem. J.* 261, 913-920.

Luminescent Substrates	Unit Size	Cat. No.
D-Luciferin, potassium salt	10 mg	10101
	50 mg	10101-1
	1 g	10101-2
D-Luciferin, sodium salt	10 mg	10102
	50 mg	10102-1
	1 g	10102-2
Coelenterazine native	50 ug	10110
	250 ug	10110-2
	1 mg	10110-1
Coelenterazine cp	50 ug	10112
	250 ug	10112-2
	1 mg	10112-1
Coelenterazine f	50 ug	10114
	250 ug	10114-2
	1 mg	10114-1
Coelenterazine h (2-(4-Dehydroxy) coelenterazine)	50 ug	10111
	250 ug	10111-2
	1 mg	10111-1

Luminescent Substrates	Unit Size	Cat. No.
Coelenterazine hcp	50 ug	10113
	250 ug	10113-2
	1 mg	10113-1
Coelenterazine i	50 ug	10121
	250 ug	10121-2
	1 mg	10121-1
Coelenterazine ip	50 ug	10116
	250 ug	10116-2
	1 mg	10116-1
Coelenterazine n	50 ug	10115
	250 ug	10115-2
	1 mg	10115-1
Coelenterazine fcp	50 ug	10117
	250 ug	10117-2
	1 mg	10117-1
Coelenterazine 400a (also known as DeepBlue C™)	50 ug	10125
	250 ug	10125-2
	1 mg	10125-1

Luminescent Substrates	Unit Size	Cat. No.
Coelenterazine, 2-methyl analog (Methyl Coelenterazine)	50 ug	10122
	1 mg	10122-1
Coelenterazine Sampler Kit	25 ug each	10123
Coelenterazine native, cp, f, fcp, h, hcp, i, ip, and n		
Aquaphile™ Coelenterazine Native	50 ug	10126-50ug
	100 ug	10126-100ug
	5 x 100 ug	10126
Aquaphile™ Coelenterazine h	50 ug	10127-50ug
	100 ug	10127-100ug
	5 x 100 ug	10127