CF® Dyes

Next-Generation Fluorescent Dyes

biotium

CF® Dyes Quick Reference Table

Visible spectrum / Far-red / Near-infrared

Dye	Page	Ex/Em (nm)	Excitation	Replacement for	Features and applications
CF®350	6	347/448	UV	Alexa Fluor® 350, AMCA, DyLight® 350	• Brightest blue fluorescent conjugates for 350 nm excitation • Highly water-soluble and pH-insensitive
CF®405S	6	404/431	405 nm	Alexa Fluor® 405, Cascade Blue™, DyLight® 405	• Brighter signal due to better compatibility with common instruments
CF®405M	6	408/452	405 nm	BD Horizon™ V450, eFluor® 450, Pacific Blue™	• More photostable than Pacific Blue™, with less green spill-over • Excellent choice for super-resolution imaging by SIM
CF®405L	6	395/545	405 nm	Pacific Orange™	• 405 nm excitable orange fluorescent dye for multicolor detection
CF®430	6	426/498	405 nm	Pacific Green™, BD Horizon™ V500, Krome Orange™	• Photostable 405 nm excitable green dye • Perfect match for the CFP filter set
CF®440	6	440/515	405 nm	Alexa Fluor® 430	• Photostable 405 nm excitable green dye
CF®450	7	450/538	405 nm	Unique dye	• Unique violet-excitable green dye
CF®488A	7	490/515	488 nm	ATTO 488, Alexa Fluor® 488, Cy®2, DyLight® 488, FAM, FITC, Fluorescein	• Less charge, for lower non-specific binding • Less red spill-over than Alexa Fluor® 488 • Validated for 2-photon and TIRF
CF®503R	8	503/532	488 nm	Unique dye	• Unique green dye or multispectral detection or FRET • Photostable rhodamine-based dye
CF®514	8	516/548	488 nm	Alexa Fluor® 514	• Green dye distinguishable from 488 nm dyes by spectral unmixing
CF®532	9	527/558	532 nm	Alexa Fluor® 532, ATTO 532	• Significantly brighter than Alexa Fluor® 532
CF®535ST	9	535/568	532 nm	Unique dye for STORM**	• Orange dye designed for STORM
CF®543	10	541/560	532 to 546 nm	Alexa Fluor® 546, Tetramethylrhodamine (TAMRA)	• Significantly brighter than Alexa Fluor® 546
CF®550R	10	551/577	532 to 568 nm	Unique dye	• Unique orange/red dye for multispectral detection or FRET • Photostable rhodamine-based dye
CF®555	10	555/565	532 to 568 nm	Alexa Fluor® 555, ATTO 550, Cy®3, DyLight® 549, TRITC	• Brighter than Cy®3 • Validated in multicolor STORM
CF®568	11	562/583	532 to 568 nm	Alexa Fluor® 568, ATTO 565, Rhodamine Red	• Optimized for the 568 nm line of the Ar-Kr mixed-gas • Brighter and more photostable than Alexa Fluor® 568 • Compatible with TIRF and multicolor STORM
CF®570	12	568/591	532 to 568 nm	Alexa Fluor® 568, ATTO 565, DY-560, Rhodamine Red	• Brighter than Alexa Fluor® 568
CF®583	12	583/606	532 to 568 nm	Cy®3.5, Texas Red®	• Brighter than Cy®3.5
CF®583R	12	586/609	532 to 568 nm	Cy®3.5, Texas Red®	• Brighter than Cy®3.5 and Texas Red® • Ideal for FRET when paired with R-PE
CF®594	13	593/614	532 to 568 nm	Alexa Fluor® 594, ATTO 594, DyLight® 594, Texas Red®	• Yields the brightest conjugates among spectrally similar dyes • Extremely photostable • Validated in 2-photon microscopy
CF®594ST	13	593/614	532 to 568 nm	Unique dye for STORM	• Specifically designed for STORM
CF®620R	13	617/639	633 or 635 nm	LightCycler® Red 640	• Highly fluorescent dye with unique spectral properties
CF®633	14	630/650	633 or 635 nm	Alexa Fluor® 633, Alexa Fluor® 647, Cy®5, DyLight® 633	• The brightest antibody conjugates among spectrally similar dyes • Far more photostable than Alexa Fluor® 647 • Compatible with super-resolution TIRF, FIONA, and gSHRImP
CF®640R	15	642/662	633 to 640 nm	Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649	• Has the best photostability among dyes with Cy®5-like spectra • Yields highly fluorescent protein conjugates • Compatible with TIRF and FLImP super-resolution techniques
CF®647	16	650/665	633 to 640 nm	Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649	• Brighter than Cy®5 • Compatible with multicolor super-resolution imaging by STORM
CF®660C	17	667/685	633 to 640 nm	Alexa Fluor® 660	• Much brighter and more photostable than Alexa Fluor® 660 • Compatible with multicolor super-resolution imaging by STORM
CF®660R	17	663/682	633 to 640 nm	Alexa Fluor® 660	• Brighter than Alexa Fluor® 660 • The most photostable 660 nm dye
CF®680	18	681/698	680 or 685 nm	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	• The brightest among spectrally similar 680 nm dyes • Validated in multicolor STORM and 3D super-resolution imaging • Compatible with LI-COR® Odyssey® System
CF®680R	18	680/701	680 or 685 nm	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	• The most photostable 680 nm dye • Suitable for labeling nucleic acids and small biomolecules • Compatible with LI-COR® Odyssey® System • Validated for 2-photon, STED, & single molecule spectroscopy
CF®700	19	695/720	680 or 685 nm	Alexa Fluor® 700, DyLight® 700	• Exceptionally bright and stable • Patented PEGDye™ dye for superior performance
CF®750	19	755/777	680 or 685 nm	Alexa Fluor® 750, Cy®7, DyLight® 750, IRDye® 750	• Exceptionally bright and photostable • Patented PEGDye™ dye for superior performance • Validated in photoacoustic imaging and STORM
CF®770	19	770/797	785 nm	DyLight® 800, IRDye® 800CW, ZW800-1	• Exceptionally bright and stable • Patented PEGDye™ dye for superior performance • Compatible with LI-COR® Odyssey® System
CF®790	19	784/806	785 nm	Alexa Fluor® 790	• Exceptionally bright and stable • Patented PEGDye™ dye for superior performance
CF®800	19	797/816	785 nm	Spectrally similar to Indocyanine green	• Unique long wavelength near-infrared dye • Patented PEGDye™ dye for superior performance
CF®820	19	822/835	785 nm	DY-820	• Exceptionally bright and stable • Patented PEGDye™ dye for superior performance

See page 4 for CF® dye applications at a glance.

CF® Dyes Technology Overview

Next-Generation Fluorescent Dyes

CF® dyes are a series of highly water-soluble fluorescent dyes spanning the visible and near-infrared (IR) spectrum for labeling biomolecules, especially proteins and nucleic acids. Developed by scientists at Biotium using new breakthrough chemistries, CF® dyes rival or exceed the quality of other commercial dyes, such as Alexa Fluor® dyes, due to several novel features.

Novel Rhodamine Chemistry

Rhodamine dyes are known for their excellent photostability and good fluorescence quantum yield; consequently several of the Alexa Fluor® dyes bear the rhodamine core structure. Unfortunately, traditional rhodamine chemistry makes it difficult to extend the fluorescence wavelength into the far-red region and even more challenging to extend into the near-IR region; especially for water-soluble dyes designed for bioconjugation. Recently, Biotium scientists discovered a new way to prepare novel rhodamine dyes of any fluorescence color from green to near-IR. The new chemistry is key to overcoming these challenges and lead to the development of many of our CF dyes. The new chemistry is a key element in the development of many of our CF® dyes, which are not only bright and water-soluble but also extremely photostable.

Excellent Labeling Efficiency

Reactive dyes for bioconjugation are generally susceptible to hydrolysis, which can cause problems for shipping, handling and storage, and result in lower labeling efficiency. Heavily sulfonated dyes, such as the Alexa Fluor® , IRDye®, and DyLight® dyes are particularly hygroscopic, worsening the hydrolysis problem. For example, the percent of active Alexa Fluor® 488 succinimidyl ester (SE) could be well below 50% by the time of application (according to the Alexa Fluor® 488 Microscale Labeling Kit product information sheet, provided by Thermo Fisher Scientific). In contrast, all of Biotium's amine-reactive CF® dyes have a relatively stable form of SE, which is more resistant to hydrolysis than the SE on many of the Alexa Fluor® dyes. Accordingly, CF® dye SE products generally give consistently higher labeling efficiency, thus providing users better results at a better value.

Mix-n-Stain™ Antibody Labeling Technology

Biotium has developed a breakthrough antibody labeling technology with CF® dye Mix-n-Stain™ antibody labeling kits. With this technology, you merely need to mix your antibody with the reaction buffer and the CF® dye provided in the kit. In 30 minutes, you will have an optimally labeled CF® dye-antibody conjugate ready for immunostaining. The labeling technology provides unprecedented convenience for antibody labeling. Mix-n-Stain™ labeled antibodies can be used for multicolor immunostaining, allowing staining with multiple primary antibodies from the same host species when pre-labeled primary antibodies are not available.

Unrivaled Near-Infrared Dyes

Near-IR dyes are typically much larger in size than dyes in the visible range. The large size often results in serious problems of low dye solubility, dye aggregation/quenching, and poor fluorescence quantum yield. To overcome the problems, many commercial near-IR dyes, such as the near-IR Alexa Fluor®, IRDye®, and DyLight® dyes, are prepared by placing a number of negatively charged sulfonate group on the dyes. While sulfonation improves dye solubility and fluorescence quantum yield to some degree, it creates another even more serious problem: non-specific binding of the bioconjugates prepared from the dyes. For example, conjugation to a highly negatively charged dye can dramatically alter an antibody's isoelectric point, which is essential for maintaining specific antibody-antigen interaction (for examples, see page 19, Figure 3 and page 31, Figure 2).

With this insight, Biotium scientists devised a revolutionary new approach to near-IR dye design using our patented polyethylene glycol dye modification, or pegylated dye chemistry. Dye pegylation offers several key benefits for dye performance:

- Increases dye solubility without adding charges
- Shields any existing charges on the dye
- Reduces dye aggregation and self-quenching on conjugates for brighter fluorescence
- Increases both thermal and photostability of the dye
- Perfectly suited for *in vivo* imaging; pegylated dye modification is known to reduce protein immunogenicity and improve biocompatibility

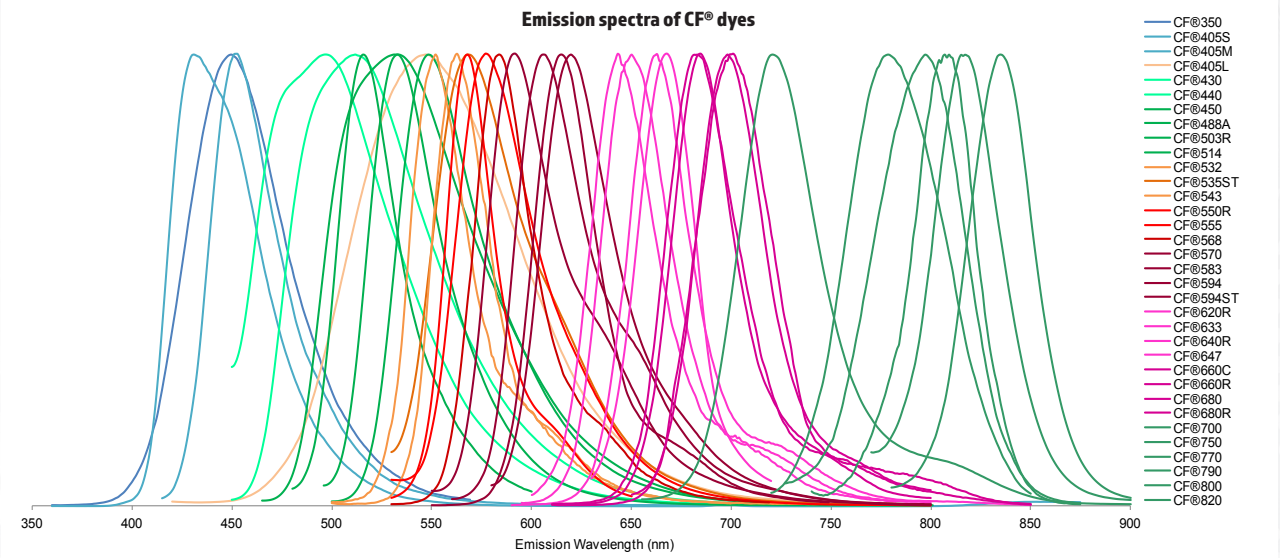
These features, along with a large and growing selection of available wavelengths, make CF® dyes the industry leaders in near-infrared dye technology. See pages 18-19 to learn more about near-infrared CF® dyes.

CF® Dyes for Super-Resolution Microscopy

Recent publications comparing synthetic dyes for super-resolution imaging have shown CF® dyes give the best performance for multiple methods. The superior brightness, photostability, and photochemical switching properties of certain CF® dyes are ideal for 3-D SIM, 3-D STORM, and other super-resolution and single-molecule imaging techniques. See page 21 for more information.

Multicolor Flexibility

Biotium currently offers more than 30 CF® dyes, with additional colors in development. The CF® dye product line includes reactive dyes with a full selection of functional groups (page 24), easy-to-use labeling kits (page 23), CF® dye-labeled primary and secondary antibodies (pages 26-29), and many other CF® dye conjugates such as toxins, tracers, ligands, and nucleotides (page 25).



CF® dyes and conjugates have been cited in hundreds of publications, with new articles published every day.
Visit www.biotium.com to download a list of selected references. See page 21 for dyes validated for super-resolution and 2-photon imaging.

Dyes At a Glance: Select the Right Dye for Your Application

Use the Spectra Viewer at www.biotium.com to find the best CF® dyes to pair with fluorescent proteins & commonly used probes.

Bright
For flow, microarray, & other applications where photobleaching isn't a concern

- CF®405S 404/431 nm
- CF®488A 490/515 nm
- CF®532 527/558 nm
- CF®543 541/560 nm
- R-PE 496-565/578 nm
- CF®555 555/565 nm
- CF®568 562/583 nm
- CF®594 593/614 nm
- CF®633 630/650 nm
- CF®640R 642/662 nm
- APC 650/660 nm
- CF®647 650/665 nm
- RPE-CF®647T 496/665 nm
- CF®660C 667/685 nm
- PerCP 482/677 nm
- CF®680 681/698 nm
- APC-CF®750T 650/780 nm
- CF®750 755/777 nm
- CF®770 770/797 nm

Bright & photostable
For microscopy & confocal imaging

- CF®405S 404/431 nm
- CF®405M 408/452 nm
- CF®430 426/498 nm
- CF®440 440/515 nm
- CF®450 405/460 nm
- CF®488A 490/515 nm
- CF®532 527/558 nm
- CF®543 541/560 nm
- CF®568 562/583 nm
- CF®583R 586/609 nm
- CF®594 593/614 nm
- CF®633 630/650 nm
- CF®640R 642/662 nm
- CF®660R 663/682 nm
- CF®680R 680/701 nm
- CF®750 755/777 nm

405 nm-excitable
For the violet laser

- CF®405S 404/431 nm
- CF®405M 408/452 nm
- CF®405L 395/545 nm
- CF®430 426/498 nm
- CF®440 440/515 nm
- CF®450 405/460 nm

Near-infrared
Industry-leading NIR dyes for microscopy, flow, *in vivo* imaging, and WB

- CF®680 681/698 nm
- CF®680R 680/701 nm
- CF®700 695/720 nm
- CF®750 755/777 nm
- CF®770 770/797 nm
- CF®790 784/806 nm
- CF®800 797/816 nm
- CF®820 822/835 nm

Alternative spectra
For FRET, multispectral imaging, or other specialized applications

- CF®450 405/460 nm
- CF®503R 503/532 nm
- CF®514 516/548 nm
- CF®405L 395/545 nm
- CF®550R 551/577 nm
- CF®620R 617/639 nm
- CF®660C 667/685 nm
- CF®800 797/816 nm
- CF®820 822/835 nm

The Fab Four
Our go-to team for 4-color confocal

- CF®405S 404/431 nm
- CF®488A 490/515 nm
- CF®568 562/583 nm
- CF®640R 642/662 nm

Near-IR western
Best match for LI-COR Odyssey®

- CF®680 681/698 nm
- CF®770 770/797 nm

STED

- CF®405M 408/452 nm
- CF®488A 490/515 nm
- CF®680R 680/701 nm

Photoacoustic imaging

- CF®750 755/777 nm

TIRF

- CF®488A 490/515 nm
- CF®568 562/583 nm
- CF®633 630/650 nm
- CF®640R 642/662 nm

STORM

- CF®488A 490/515 nm
- CF®535ST 527/558 nm
- CF®555 555/565 nm
- CF®568 562/583 nm
- CF®594ST 593/614 nm
- CF®647 650/665 nm
- CF®660C 667/685 nm
- CF®680 681/698 nm
- CF®680R 680/701 nm
- CF®750 755/777 nm

SIM

- CF®405S 404/431 nm
- CF®405M 408/452 nm
- CF®568 562/583 nm
- CF®640R 642/662 nm

2-photon

- CF®488A 490/515 nm
- CF®594 593/614 nm
- CF®680R 680/701 nm

See page 20-21 for more information on CF® dyes for super-resolution imaging and other specialized applications.
CF® dyes are being tested in new applications all the time, visit biotium.com for the most up-to-date information.

Frequently Asked Questions (FAQs)

Question	Answer
What does the CF in CF® dyes stand for?	CF® was initially an abbreviation for "Cyanine-based Fluorescent dyes". These were the first patented CF® dyes based on cyanine dye structures. 10 years and more than two dozen dyes later, the CF® dye portfolio encompasses multiple dye core structures spanning the fluorescence spectrum from UV to near-IR. Today, we believe "CF" more aptly stands for Clear Fluor: dyes that produce superior signal-to-noise.
What are pegylated dyes?	Dye pegylation is one of Biotium's patented dye technologies that improves solubility and brightness of near-infrared dyes without introducing excess negative charge, making our near-IR CF® dyes industry leaders. See page 3 for more information on pegylated dye technology; see pages 18-19 to learn more about near-IR CF® dyes.
What are the chemical structures of CF® dyes?	The exact chemical structures of CF® dyes are currently confidential but will be fully disclosed at a later stage when pending patents become granted. In general terms, the structure of a CF® dye may be divided into two parts: a) dye core structure (i.e. the aromatic ring skeleton that defines the dye's color or absorption/emission wavelengths), and b) core structure-modifying elements. At present, CF® dyes bear the core structures of coumarin, pyrene, rhodamine, or cyanine dyes. Blue fluorescent CF® dyes are based on a coumarin or pyrene dye core structure, while green to near-IR CF® dyes are based on either cyanine or rhodamine dye core structures. Core structure-modifying elements refer to various chemical attachments to the core structure and are a key aspect of the CF® dye invention that makes CF® dyes superior to other commercial dyes.
What are the quantum yields of CF® dyes?	The quantum yield of a fluorescent dye can vary widely depending on the dye's micro-environment and if the dye is attached to a protein or other molecule. A good way to compare the relative quantum yields of different dyes is to plot the total fluorescence of the labeled proteins as a function of degree of labeling by the dyes, as we have done with CF® dyes and other commercial dyes in the dye description pages in this guide.
How stable are CF® dyes?	There are three aspects to dye stability: • **Chemical stability:** CF® dyes bear the core structures of coumarin, pyrene, rhodamine, or cyanine dyes; all of which are known to have excellent chemical stability. In general, the dyes are far more stable than the antibodies or other biomolecules they label. CF® dyes are also stable enough for labeled nucleic acids to be used in PCR or nucleic acid hybridization, where high temperature is involved. • **Reactive group stability:** Reactive CF dyes have a chemically reactive group for bioconjugation. Among the various reactive groups, only amine-reactive succinimidyl ester (SE) and thiol-reactive maleimide groups are susceptible to hydrolysis and therefore are moisture-sensitive. CF® dye SE products are relatively more stable than other SE dyes. This is because CF® SE dyes are derived from aliphatic carboxylic groups, which results in a more stable SE form, while other SE dyes usually are derived from aromatic carboxylic acid groups that yield a less stable SE form. • **Photostability:** This refers to the dye's ability to withstand photobleaching. Photobleaching is mainly a concern when dyes are subjected to intense illumination for an extended period of time, such as during confocal microscopy. Among the four types of core structures, rhodamine is the most photostable, followed by cyanine, pyrene, and coumarin cores. The structure-modifying groups and the way they are attached to the dye cores are a key innovative aspect of CF® dye technologies that contributes to the superior photostability of CF® dyes over that of other dyes. In general, rhodamine-based CF® dyes, whose wavelengths range from green to the near-IR region, offer the best photostability, making these dyes ideal for microscopy applications.
Are CF® dyes sensitive to pH?	CF® dyes are chemically stable within the range of at least pH 2 to pH 11. The fluorescence of most CF® dyes is relatively insensitive to pH, except for that of CF®405M, CF®568, CF®620R, and CF®633. The fluorescence of these four CF® dyes becomes weaker when pH drops below 4.5.
Are CF® dyes fixable?	CF® dyes can tolerate formaldehyde fixation. However, whether a CF® dye-labeled probe is fixable will depend on the fixability of the probe itself. Proteins with free amine groups that bind other proteins generally are formaldehyde-fixable.
What is the difference between CF®405S, CF®405M, and CF®405L?	All three of these dyes can be excited by the 405 nm laser (or UV mercury lamp). They differ in their emission wavelengths. CF®405S has the shortest blue fluorescence emission at 431 nm, while CF®405M has a longer wavelength blue fluorescence emission at 452 nm. CF®405L has orange fluorescence emission at 545 nm. We recommend choosing the dye that best fits your instrument's detection settings (see pages 6-7 for more information).
For several CF® dye colors, there is an R form and a C form, both having similar absorption and emission spectra. In such a case, which of the two CF® dyes should I choose?	Rhodamine-based CF® dyes (designated R) generally have better photostability but weaker fluorescence than their cyanine-based equivalents (designated C). Therefore, rhodamine-based near-IR CF® dyes are a better choice for microscopy, while cyanine-based CF® dyes are more ideal for flow cytometry, western blotting, and other applications where photobleaching is less of a concern. Another factor to consider is the size of the dyes. Some of the cyanine-based near-IR CF® dyes are much larger than the rhodamine-based equivalents. For antibody labeling, either version of the CF® dyes is suitable. However, for applications where the dye size may cause a steric problem, the smaller dye may be a better choice.
How soluble are CF® dyes?	CF® dyes are highly water-soluble (>100 mg/mL). They are also very soluble in other polar solvents, such as DMSO, DMF, methanol, and ethanol. However, CF® dyes are poorly soluble or insoluble in non-polar solvents.
What are the charges on CF® dyes?	Most CF® dyes carry 1-2 negative charges while some cyanine-based near-IR CF® dyes carry 3-4 negative charges. However, the more negatively charged CF® dyes have unique structural features that shield the biomolecules from the negative charges; such that the biomolecules (such as antibodies) do not lose specificity due to excess negative charge.
Can CF® dyes be used for STORM, STED, SIM, or TIRF?	Many of our CF® dyes have been validated in multiple super-resolution techniques. Biotium also offers dyes specifically designed for STORM imaging. See pages 20-21 for more information.
Can CF® dyes be used for 2-photon microscopy?	Some CF® dyes have been validated for 2-photon excitation. See pages 20-21 for more information.
What are the major applications of CF® dyes?	CF® dyes are ideal for protein labeling because of their high water solubility, which reduces fluorescence quenching. They are also useful for labeling oligonucleotides that require multiple copies of a dye for maximal fluorescence, such as the preparation of FISH probes, where water-soluble dyes can minimize fluorescence quenching. Finally, CF® dyes make excellent polar tracers that can be used for visualizing the morphology or long-term tracing of neurons. Several CF® dyes have been validated in specialized applications, including spectral flow cytometry, SIM, TIRF, STORM, and other super-resolution imaging techniques, as well as photoacoustic imaging and 2-photon microscopy. See pages 20-21 for more information about CF® dyes in super-resolution and other specialized imaging applications.

CF® 350

A bright UV-excitable blue fluorescent dye

Technical Summary

Abs/Em maxima: 347/448 nm

Extinction coefficient: 18,000

Molecular weight: ~ 496

Excitation source: UV

Replaces: Alexa Fluor® 350, AMCA, DyLight® 350

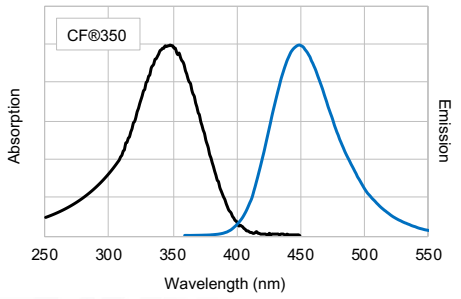


Figure 1. Absorption and emission spectra of CF®350 goat anti-mouse conjugate in PBS.

Features

- Brighter and more photostable than AMCA
- Direct replacement for Alexa Fluor® 350
- Highly water-soluble and pH-insensitive

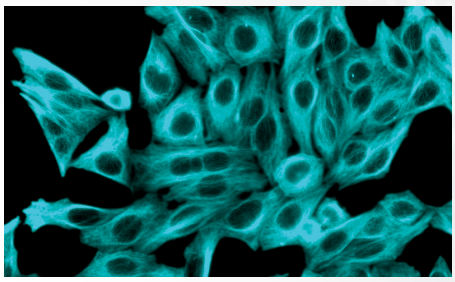


Figure 2. HeLa cells stained with mouse anti-tubulin antibody and CF®350 goat anti-mouse IgG (cyan).

CF®405S and CF®405M

Improved brightness and photostability for the 405 nm laser line

Technical Summary

CF®405S

Abs/Em maxima: 404/431 nm

Extinction coefficient: 33,000

Molecular weight: ~ 1,169

Excitation laser line: 405 nm

Replaces: Alexa Fluor® 405, Cascade Blue™, DyLight® 405

CF®405M

Abs/Em maxima: 408/452 nm

Extinction coefficient: 41,000

Molecular weight: ~ 503

Excitation laser line: 405 nm

Replaces: Pacific Blue™, BD Horizon™ V450

Features

- CF®405S: Brighter than Alexa Fluor® 405 (Fig. 2)
- CF®405M: More photostable than Pacific Blue™, with less spill-over in the green channel
- Validated for super-resolution imaging by SIM (see p. 21)

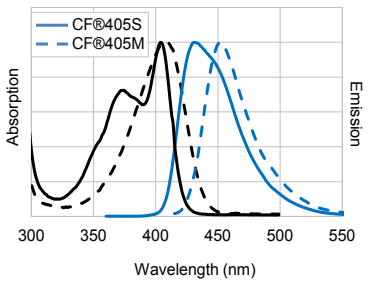
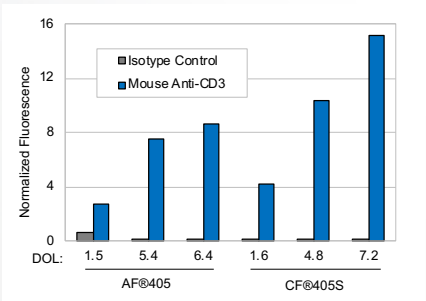


Figure 1. Absorption and emission spectra of CF®405S and CF®405M goat anti-mouse conjugates in PBS.

Figure 2. Intracellular staining of Jurkat cells was performed with mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugated to Alexa Fluor® 405 (AF405) or CF®405S. Fluorescence was analyzed on a BD LSRII flow cytometer with 405 nm excitation and 450/50 nm emission filter. Bars represent geometric mean fluorescence.



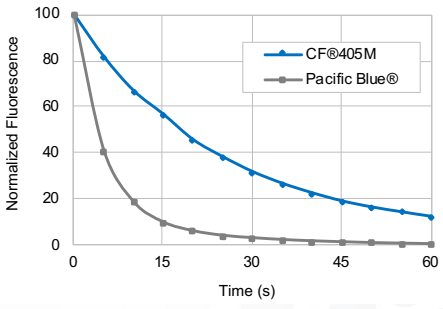


Figure 3. Relative photostability of CF®405M and Pacific Blue™. CF®405M and Pacific Blue™ dye solutions were continuously exposed to mercury arc lamp microscope excitation with a DAPI filter set. Images were captured every 5 seconds for one minute. Fluorescence intensity was normalized to time 0.

CF®405L

A 405 nm-excitable dye with orange fluorescence emission

Technical Summary

Abs/Em maxima: 395/545 nm

Extinction coefficient: 24,000

Molecular weight: ~ 1573

Excitation laser line: 405 nm

Replaces: Pacific Orange™

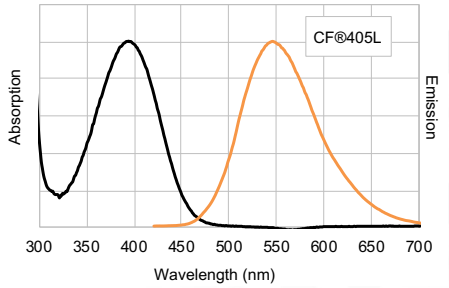


Figure 1. Absorption and emission spectra of CF®405L goat anti-mouse conjugate in PBS.

CF®430 and CF®440

Photostable 405 nm-excitable dyes with green fluorescence

Technical Summary

CF®430

Abs/Em maxima: 426/498 nm

Extinction coefficient: 40,000

Molecular weight: ~ 429

Excitation laser line: 405 nm

Replaces: Pacific Green™, BD Horizon™ V500, Krome Orange™

CF®440

Abs/Em maxima: 440/515 nm

Extinction coefficient: 40,000

Molecular weight: ~ 716

Excitation laser line: 405 nm

Replaces: Alexa Fluor® 430

Features

- Photostable dyes suitable for microscopy
- CF®430 is a perfect match for the CFP filter set
- Suitable for flow cytometry in the AmCyan channel
- Highly water-soluble and pH-insensitive

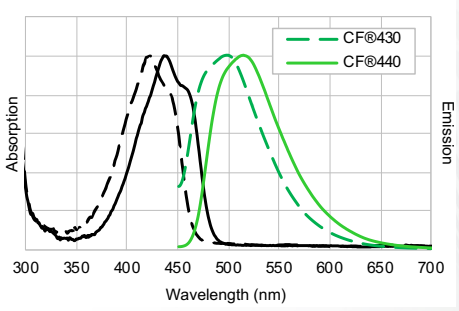


Figure 1. Absorption and emission spectra of CF®430 and CF®440 goat anti-mouse conjugates in PBS.

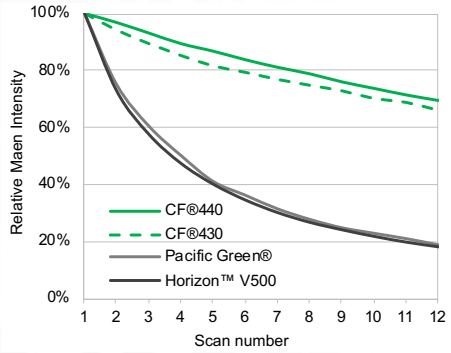


Figure 2. Relative photostability of CF®430 and CF®440 compared to spectrally-similar dyes. Cells were stained with biotinylated primary antibodies followed by streptavidin conjugates of CF®430, CF®440, Pacific Green™, or BD Horizon™ V500. Fluorescence was imaged on a Zeiss LSM700 confocal microscope in the FITC channel using 405 nm excitation. Images were acquired every 5 seconds for 12 consecutive scans of the same field of view using the same imaging settings for each dye. The mean fluorescence intensity of each image was normalized to the first scan for each dye.

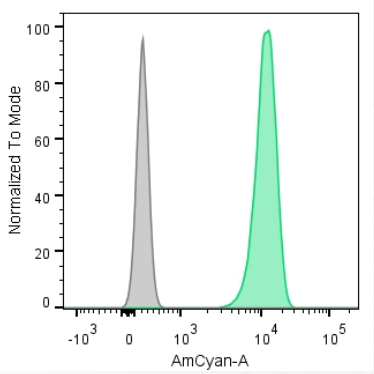


Figure 3. Flow cytometry analysis of Jurkat cells stained with isotype control (gray peak) or mouse anti-CD3 (green peak) followed by CF®430 goat anti-mouse IgG, analyzed in the AmCyan channel of a BD LSRII flow cytometer.

CF®450

CF®450

405 nm-excitable green dye with unique spectral properties

Technical Summary

Abs/Em maxima: 450/538 nm

Extinction coefficient: 40,000

Molecular weight: ~ 689

Excitation laser line: 405 nm

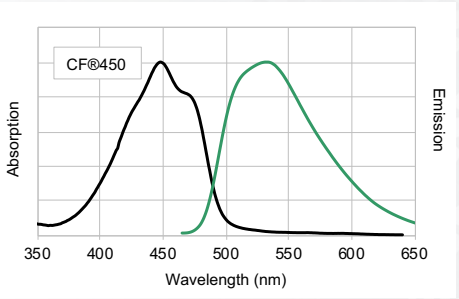


Figure 1. Absorption and emission spectra of CF®450 goat anti-mouse conjugate in PBS.

CF®488A

CF®488A

A superior green fluorescent dye

Technical Summary

Abs/Em maxima: 490/515 nm

Extinction coefficient: 70,000

Molecular weight: ~ 914

Excitation laser line: 488 nm

Replaces: Alexa Fluor® 488, DyLight® 488, fluorescein (FITC, FAM), Cy®2

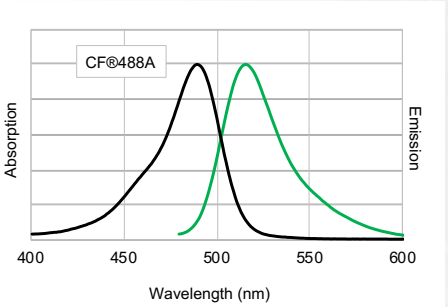


Figure 1. Absorption and emission spectra of CF®488A goat anti-mouse conjugate in PBS.

Features

- Minimally charged, for less non-specific binding than Alexa Fluor® 488
- Narrower emission spectrum for less bleed into the red channel
- Very photostable
- Compatible with STED, TIRF, and 2-photon microscopy (p. 21)

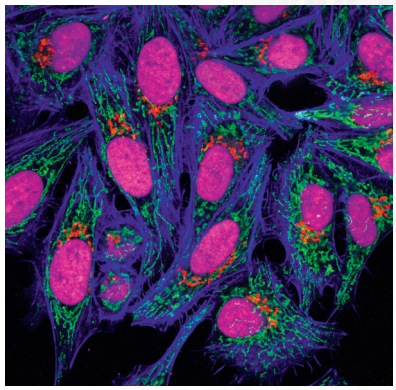


Figure 2. HeLa cells stained with rabbit anti-COXIV and CF®488A goat anti-rabbit IgG (mitochondria, green), mouse anti-Golgin 97 and CF®555 goat anti-mouse IgG (Golgi, red), CF®405M phalloidin (actin filaments, blue), and RedDot™2 (nuclei, magenta). See p. 31 for more information on RedDot™2.

CF®503R & CF®514

CF®503R & CF®514

Alternative green fluorescent dyes for spectral imaging

Technical Summary

CF®503R

Abs/Em maxima: 503/532 nm

Extinction coefficient: 90,000

Molecular weight: ~ 1100

Excitation laser line: 488 nm

Technical Summary

CF®514

Abs/Em maxima: 516/548 nm

Extinction coefficient: 105,000

Molecular weight: ~ 1216

Excitation laser line: 488 nm

Replaces: Alexa Fluor® 514

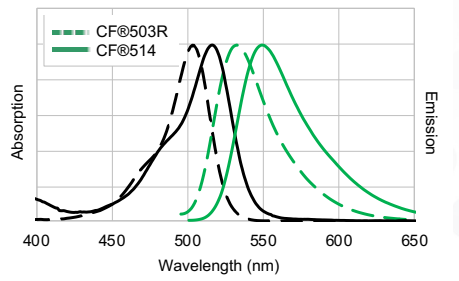


Figure 1. Absorption and emission spectra of CF®503R or CF®514 goat anti-mouse conjugates in PBS.

CF®532

A bright orange fluorescent dye for the 532 nm laser

Technical Summary

Abs/Em maxima: 527/558 nm

Extinction coefficient: 96,000

Molecular weight: ~ 685

Excitation laser line: 532 nm

Replaces: Alexa Fluor® 532, Atto 532

Features

- Designed for the 532 nm laser
- Brighter than Alexa Fluor® 532 (Fig. 2)
- Highly water-soluble and pH-insensitive

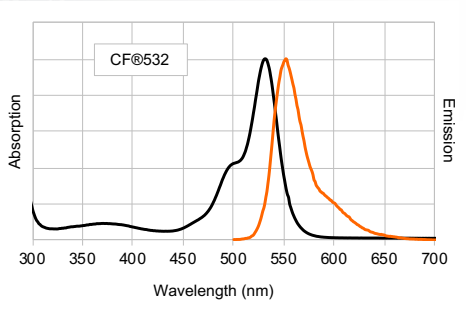


Figure 1. Absorption and emission spectra of CF®532 goat anti-mouse IgG conjugate in PBS.

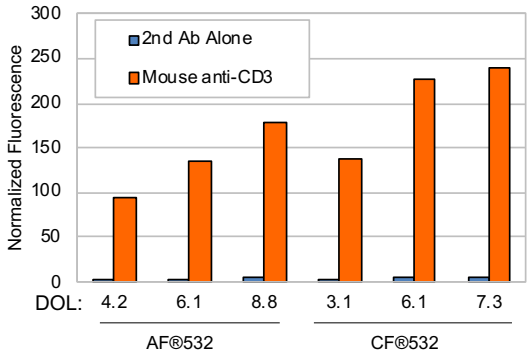


Figure 2. Flow cytometry analysis of Jurkat cells stained with Alexa Fluor® 532 (AF532) antibody or CF®532 secondary antibody conjugates. Intracellular staining was performed with mouse anti-CD3 antibody followed by goat anti-mouse IgG conjugates. Background was determined by staining with secondary antibody (2nd Ab) alone. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL2 channel. The bars represent the relative fluorescence of the geometric means of the cell populations.

CF®535ST

An orange fluorescent dye designed for STORM super-resolution imaging

Technical Summary

Abs/Em maxima: 535/568 nm

Extinction coefficient: 95,000

Molecular weight: ~ 728

Excitation laser line: 532 nm

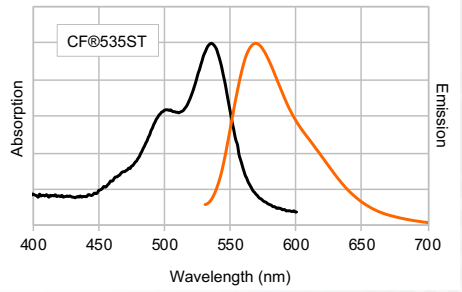


Figure 1. Absorption and emission spectra of CF®535ST goat anti-mouse IgG conjugate in PBS.

See page 21 for more information about CF® dyes for super-resolution imaging.

CF®543

CF®543

Bright orange dye ideal for the 543 nm laser

Technical Summary

Abs/Em maxima: 541/560 nm

Extinction coefficient: 100,000

Molecular weight: ~ 870

Excitation laser line: 532 to 546 nm

Replaces: Alexa Fluor® 546, TAMRA

Features

- Optimized for the 543 nm laser
- Brightest conjugates among similar dyes
- Highly water-soluble and pH-insensitive

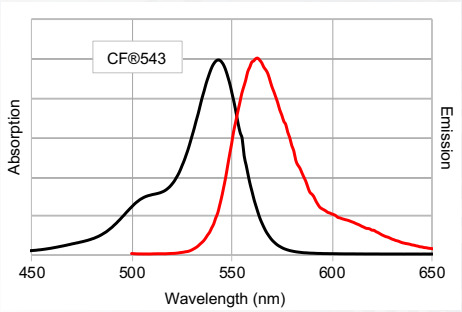


Figure 1. Absorption and emission spectra of CF®543 goat anti-mouse conjugate in PBS.

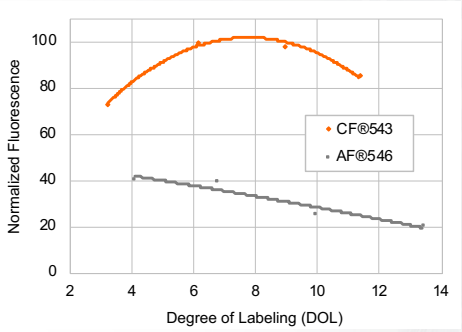


Figure 2. Relative fluorescence of CF®543 and Alexa Fluor® 546 (AF546) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

CF®550R

CF®550R

Alternative orange/red dye for spectral imaging

Technical Summary

Abs/Em maxima: 551/577 nm

Extinction coefficient: 100,000

Molecular weight: ~ 686

Excitation laser line: 532 nm or 568 nm

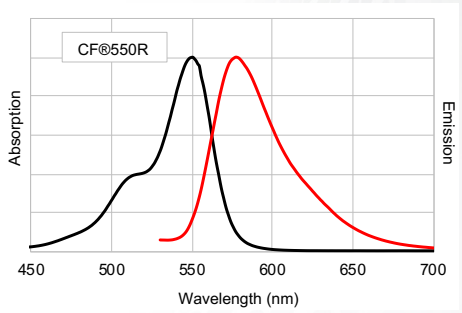


Figure 1. Absorption and emission spectra of CF®550R goat anti-mouse conjugate in PBS.

CF®555

CF®555

A bright and photostable orange-red dye

Technical Summary

Abs/Em maxima: 555/565 nm

Extinction coefficient: 150,000

Molecular weight: ~ 959

Excitation laser line: 532 nm or 568 nm

Replaces: Alexa Fluor® 555, ATTO 550, Cy®3, DyLight® 549, Rhodamine

Features

- Brighter than Cy®3
- Highly water-soluble
- Validated in multicolor STORM (see p. 21)

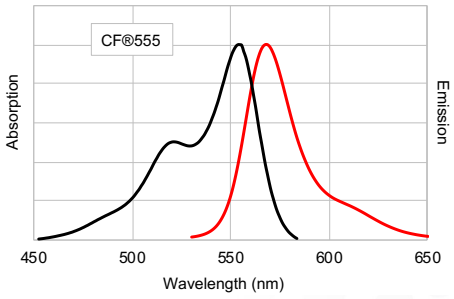


Figure 1. Absorption and emission spectra of CF®555 goat anti-mouse conjugate in PBS.

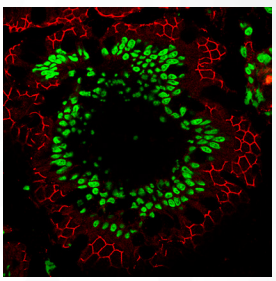


Figure 2. Rat intestine stained with CF®555 Mix-n-Stain™ labeled mouse anti-ZO1 (tight junctions, red) and NucSpot® 470 (nuclei, green). See p. 22 for more information on Mix-n-Stain™ kits.

CF®568
Outshines Alexa Fluor®568

Technical Summary

Abs/Em maxima: 562/583 nm

Extinction coefficient: 100,000

Molecular weight: ~ 714

Excitation laser line: 532 nm or 568 nm

Replaces: Alexa Fluor® 568, ATTO 565, Rhodamine Red

Features

- Much brighter antibody conjugates than Alexa Fluor® 568
- Extremely photostable
- Excellent choice for multiplexing with CF®488A and CF®640R
- Compatible with TIRF and multicolor STORM (see p. 21)

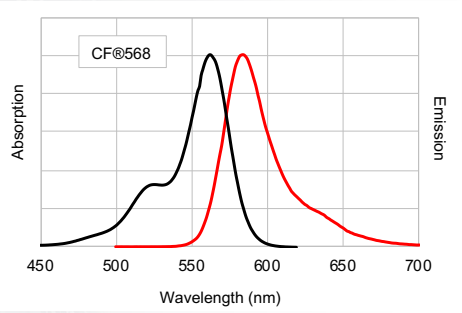


Figure 1. Absorption and emission spectra of CF®568 goat anti-mouse conjugate in PBS.

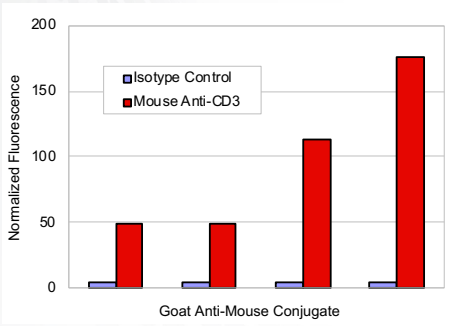


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL2 channel. Bars represent the relative fluorescence of the geometric means of the cell populations.

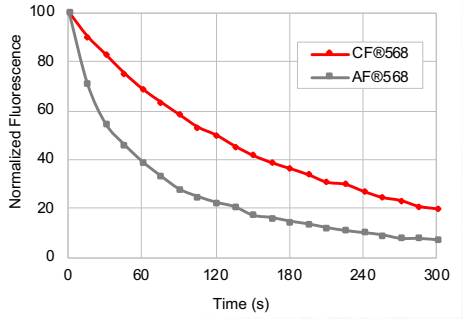


Figure 3. Photostability of CF®568 and Alexa Fluor® 568 (AF568) streptavidin conjugates. Intracellular staining of Jurkat cells was performed using anti-CD3-biotin followed by streptavidin conjugated to CF®568 or AF568. Cells were continuously exposed to mercury arc lamp microscope excitation with a Cy®3 filter set. Images were captured every 15 seconds for 5 minutes and fluorescence intensity was normalized to time 0.

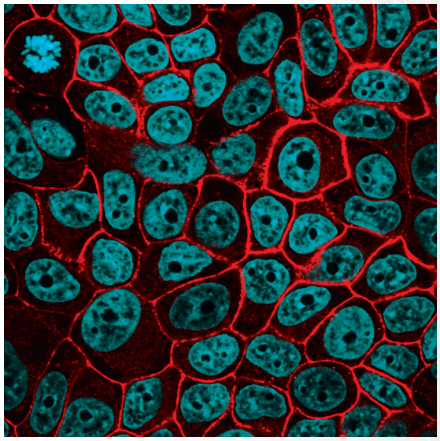


Figure 4. MCF-7 cells stained with CF®568 monoclonal anti-Ep-CAM (clone EGP40/826) at 5 ug/mL (red). Nuclei are counterstained with Hoechst 33342 (blue). See p. 26 for more information on primary antibody conjugates.

CF®570

CF®570

Red fluorescent dye with superior brightness

Technical Summary

Abs/Em maxima: 568/591 nm

Extinction coefficient: 150,000

Molecular weight: ~ 2998

Excitation laser line: 561 to 568 nm

Replaces: Alexa Fluor® 568, ATTO 565, DY-560, Rhodamine Red

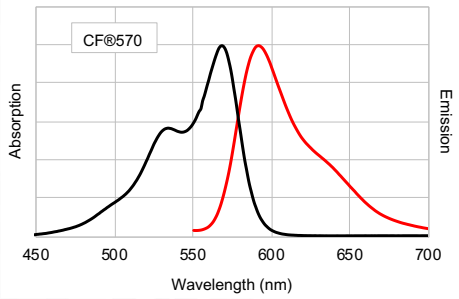


Figure 1. Absorption and emission spectra of CF®570 goat anti-mouse conjugate in PBS.

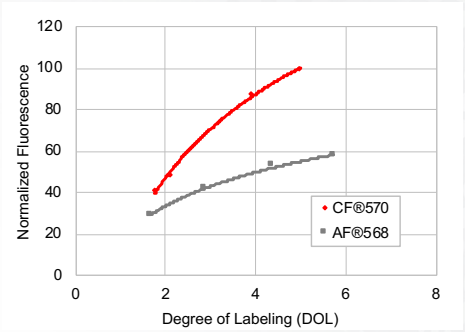


Figure 2. Relative fluorescence of CF®570 and Alexa Fluor® 568 (AF568) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

CF®583

CF®583 & CF®583R

Brighter than Cy®3.5

CF®583 Technical Summary

Abs/Em maxima: 583/606 nm

Extinction coefficient: 150,000

Molecular weight: ~ 3127

Excitation laser line: 561 to 568 nm

Replaces: Cy®3.5

CF®583R Technical Summary

Abs/Em maxima: 586/609 nm

Extinction coefficient: 100,000

Molecular weight: ~ 773

Excitation laser line: 561 to 568 nm

Spectrally similar to: Cy®3.5, Texas Red®

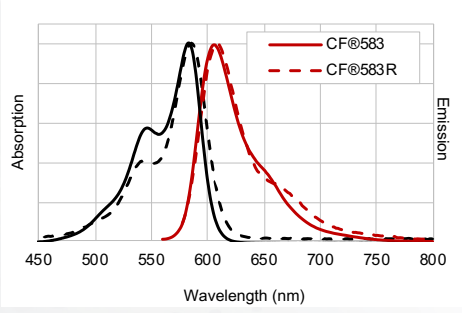


Figure 1. Absorption and emission spectra of CF®583 & CF®583R

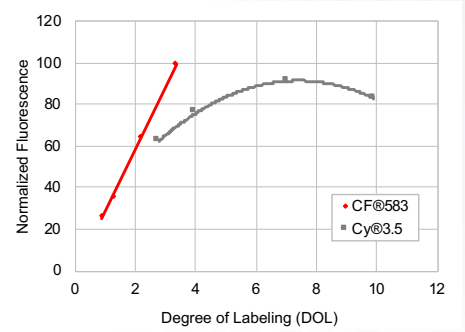


Figure 2. Relative fluorescence of CF®583 and Cy®3.5 goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

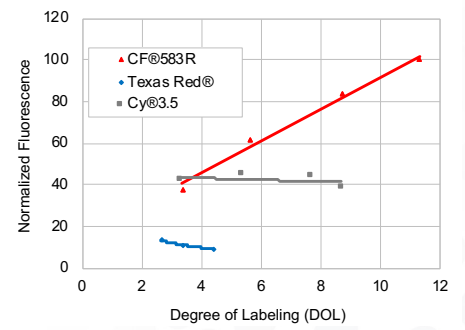


Figure 3. CF®583R produces brighter conjugates at a lower degree of labeling than Cy®3.5 and Texas Red®. Relative fluorescence of goat anti-mouse conjugates of the indicated dyes at varying degrees of labeling (DOL, or dye molecules per antibody).

CF®594 & CF®594ST

Truly the brightest deep red dye, with STORM-compatible option

Technical Summary

Abs/Em maxima: 593/614 nm

Extinction coefficient: 115,000

Molecular weight: ~ 729

Excitation laser line: 532 to 594 nm

Replaces: Alexa Fluor® 594, DyLight® 594, Texas Red®

Features

- Brightest antibody conjugates among spectrally similar dyes, with excellent photostability
- Compatible with 2-photon microscopy (see p. 21)
- CF®594ST matches CF®594 spectrally, but is compatible with STORM imaging (see p. 21)

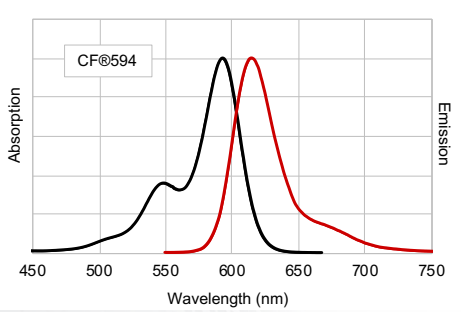


Figure 1. Absorption and emission spectra of CF®594 goat anti-mouse conjugate in PBS.

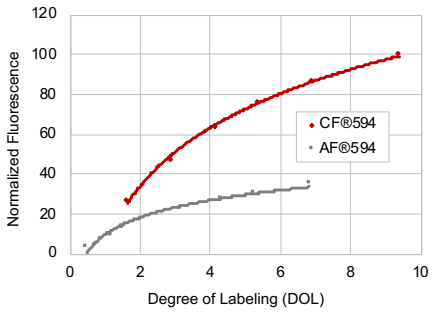


Figure 2. Relative fluorescence of CF®594 and Alexa Fluor® 594 (AF594) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

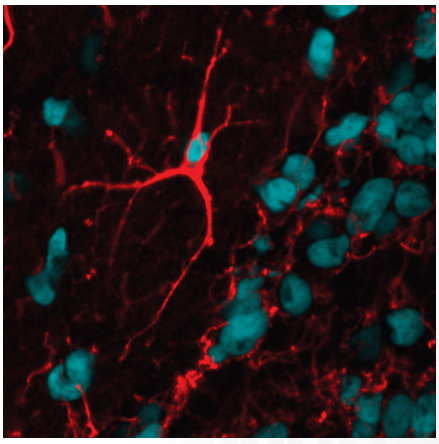


Figure 3. Glial cells in frozen section of rat brain stained with rabbit anti-GFAP antibody and CF®594 goat anti-rabbit IgG (red). Nuclei are stained with RedDot™2 (pseudocolored cyan). Mounted with Everbrite™ Mounting Medium. See p. 31 for more information on RedDot™2 and EverBrite™ Mounting Medium.

CF®620R

A bright and photostable far-red dye

Technical Summary

Abs/Em maxima: 617/639 nm

Extinction coefficient: 115,000

Molecular weight: ~ 738

Excitation laser line: 633 nm or 635 nm

Spectrally similar to: LightCycler® Red 640

Features

- Highly fluorescent and extremely photostable
- Absorption/emission at 617/639 nm for use in FRET or other specialized applications

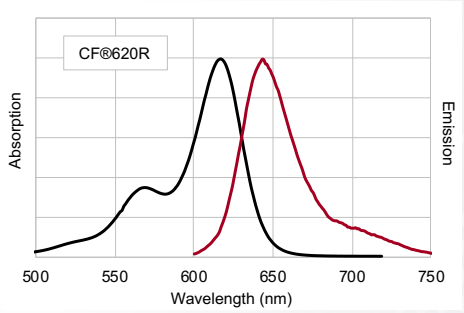


Figure 1. Absorption and emission spectra of CF®620R free acid in PBS.

CF®633

The best dye for 633/635 laser lines

Technical Summary

Abs/Em maxima: 630/650 nm

Extinction coefficient: 100,000

Molecular weight: ~ 821

Excitation laser line: 633 nm or 635 nm

Replaces: Alexa Fluor® 633, Alexa Fluor® 647, Cy®5, DyLight® 633, DyLight® 649

Features

- Yields the brightest antibody conjugates among spectrally similar dyes
- Far more photostable than Alexa Fluor® 647
- Compatible with TIRF, FIONA, and gSHRImP super-resolution imaging methods (see p. 21)

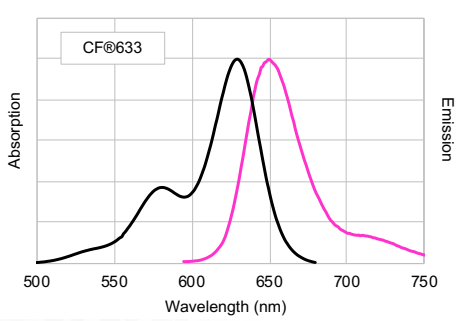


Figure 1. Absorption and emission spectra of CF®633 goat anti-mouse conjugate in PBS.

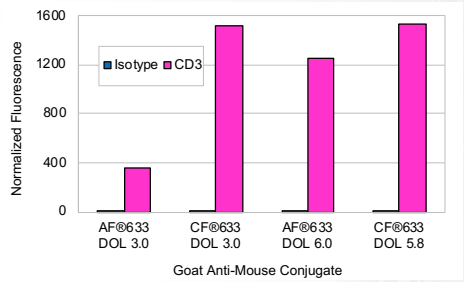


Figure 2. CF®633 yields the brightest far-red conjugates. Jurkat cells were stained with mouse anti-CD3 or isotype control antibody, followed by goat anti-mouse conjugates with varying degrees of labeling (DOL, or dye molecules per antibody). Fluorescence was measured in the APC channel of a BD FACSCalibur™ flow cytometer; bars represent geometric mean fluorescence.

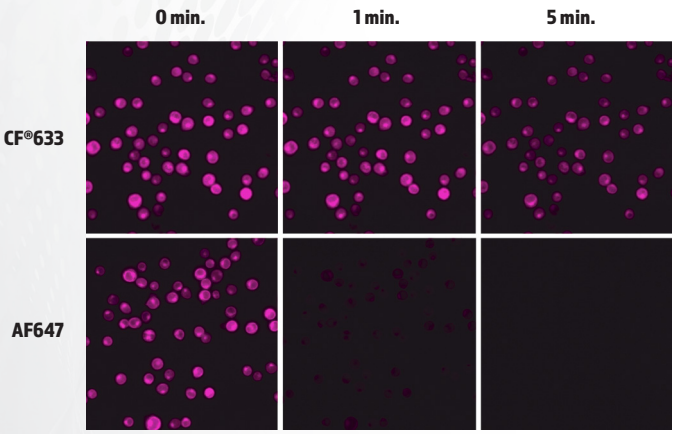


Figure 3. Relative photostability of CF®633 and Alexa Fluor® 647 (AF647) goat anti-mouse conjugates. Jurkat cells were fixed, permeabilized, and stained with rabbit anti-CD3 followed by CF®633 or Alexa Fluor® 647 goat anti-rabbit IgG conjugates. Cells were imaged using a mercury arc lamp microscope equipped with a Cy®5 filter set and CCD camera. Sequential images were captured at 0, 1, and 5 minutes.

CF®640R

A highly photostable far-red dye for the 640 nm laser

Technical Summary

Abs/Em maxima: 642/662 nm

Extinction coefficient: 105,000

Molecular weight: ~ 832

Excitation laser line: 633 to 640 nm

Replaces: Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649

Features

- Best photostability among Cy®5-like dyes
- Yields highly fluorescent protein conjugates
- Compatible with TIRF and FLImP super-resolution microscopy (see p. 21)

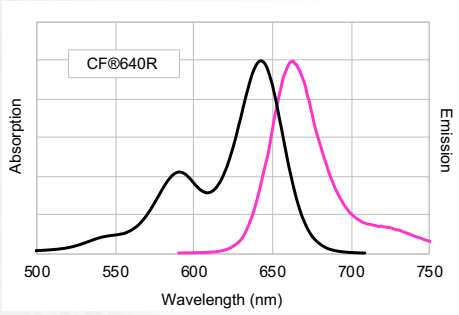


Figure 1. Absorption and emission spectra of CF®640R goat anti-mouse conjugate in PBS.

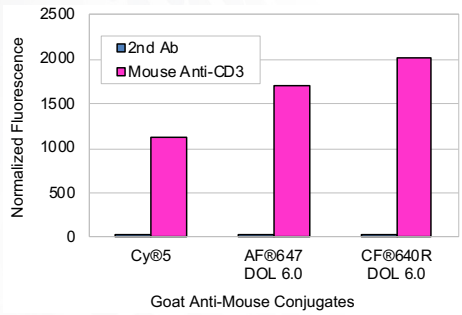


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel. Bars represent the relative fluorescence of the geometric means of the cell populations.

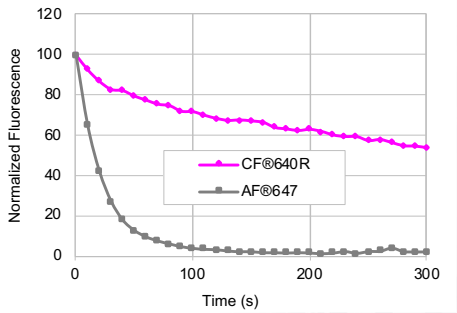


Figure 3. Relative photostability of CF®640R and Alexa Fluor® 647 (AF647). HeLa cells were stained with anti-tubulin antibody conjugates of each dye. Cells were continuously illuminated by a mercury arc lamp microscope and sequential images were captured at 0, 1, and 3 minutes. Mean fluorescence was normalized to time 0.

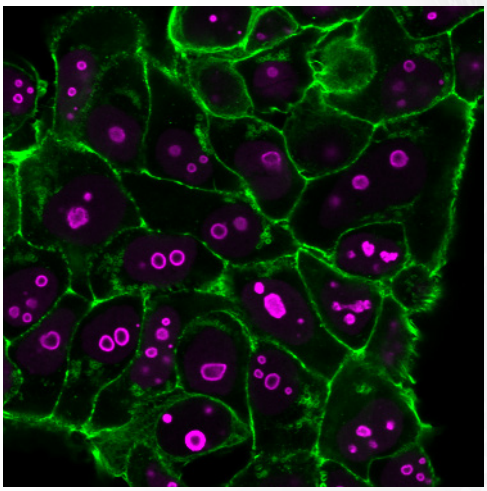


Figure 4. MCF-7 cells stained with CF®640R monoclonal anti-Cyclin B1 (clone CCNB1/1098) at 5 ug/mL (magenta). Actin filaments are stained with CF®488A phalloidin (green). See p. 26 for more information on primary antibody conjugates.

CF®647

A highly fluorescent far-red dye

Technical Summary

Abs/Em maxima: 650/665 nm

Extinction coefficient: 240,000

Molecular weight: ~ 1058

Excitation laser line: 633 to 640 nm

Replaces: Cy®5, Alexa Fluor® 647, DyLight® 649

Features

- Brighter than Cy®5
- Highly water-soluble and pH-insensitive
- Validated in multi-color STORM imaging (see p. 21)

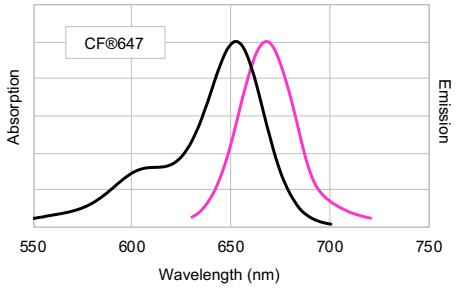


Figure 1. Absorption and emission spectra of CF®647 goat anti-mouse conjugate in PBS.

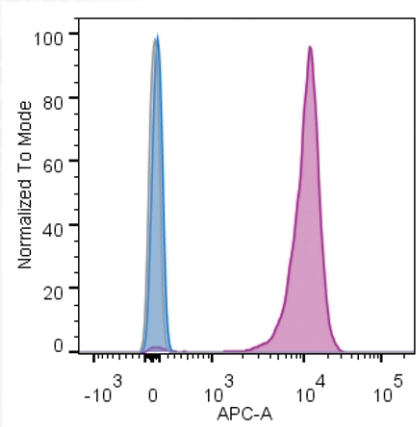


Figure 2. Intracellular staining of Jurkat cells with CF®647 monoclonal anti-nucleolin (clone 365-2) (pink) or CF®647 IgG1 isotype control (blue) at 1 ug/tube, compared to unstained cells (yellow). Cells were analyzed in the APC channel of a BD LSRII flow cytometer. See p. 26 for more information on primary antibody conjugates.

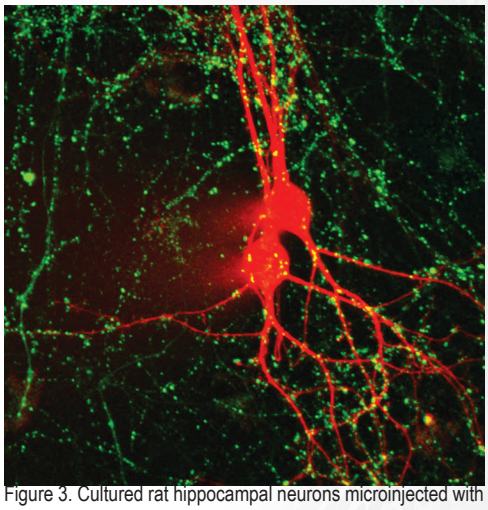


Figure 3. Cultured rat hippocampal neurons microinjected with CF®647 hydrazide (red) and stained with SynaptoGreen™ C4 (FM®1-43) (green, synaptic vesicles). Image courtesy of Professor Guosong Liu, Tsinghua University, Beijing, China.

CF®660C and CF®660R

Superior alternatives to Alexa Fluor® 660

Technical Summary

CF®660C

Abs/Em maxima: 667/685 nm

Extinction coefficient: 200,000

Molecular weight: ~ 3112

Excitation laser line: 633 to 640 nm

Replaces: Alexa Fluor® 660, APC

CF®660R

Abs/Em maxima: 663/682 nm

Extinction coefficient: 100,000

Molecular weight: ~ 888

Excitation laser line: 633 to 640 nm

Replaces: Alexa Fluor® 660, APC

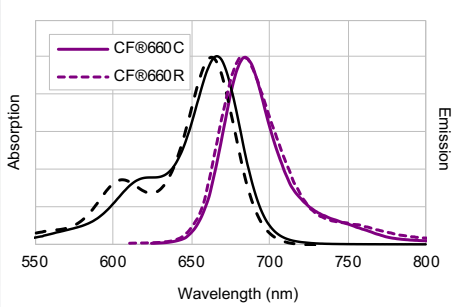


Figure 1. Absorption and emission spectra of CF®660C and CF®660R goat anti-mouse conjugates in PBS.

CF®660C Features

- Much brighter and more photostable than Alexa Fluor® 660
- Compatible with multicolor super-resolution imaging by STORM (see p. 21)

CF®660R Features

- Brighter than Alexa Fluor® 660
- Unrivaled photostability among spectrally similar dyes

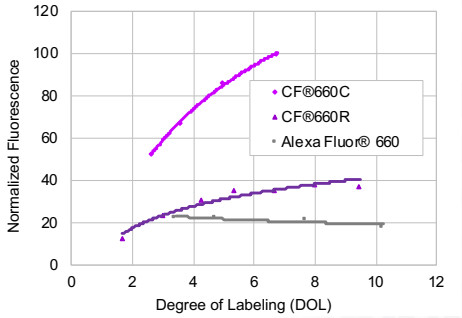


Figure 2. Relative fluorescence of CF®660, CF®660R, and Alexa Fluor® 660 (AF660) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

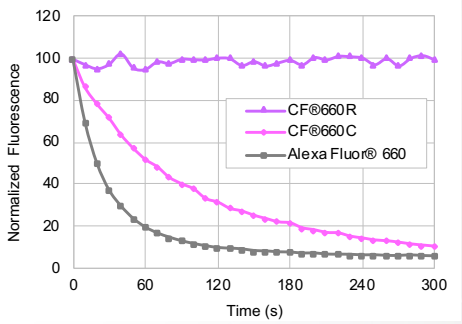


Figure 3. Relative photostability of CF®660C, CF®660R, and Alexa Fluor® 660 (AF660) conjugates. HeLa cells were stained with mouse anti-tubulin followed by CF®660C, CF®660R or AF660 goat anti-mouse IgG conjugates. Cells were continuously exposed to mercury arc lamp microscope excitation using a Cy®5 filter set. Images were captured every 10 seconds for five minutes and fluorescence intensity was normalized to time 0.

CF®680 and CF®680R

Two outstanding 680 nm-excitable dyes

Technical Summary

CF®680

Abs/Em maxima: 681/698 nm

Extinction coefficient: 210,000

Molecular weight: ~ 3241

Excitation laser line: 680 nm or 685 nm

Replaces: Alexa Fluor® 680, Cy®5.5, IRDye® 680

CF®680R

Abs/Em maxima: 680/701 nm

Extinction coefficient: 140,000

Molecular weight: ~ 912

Excitation laser line: 680 nm or 685 nm

Replaces: Alexa Fluor® 680, Cy®5.5, IRDye® 680

CF®680 Features

- The brightest among spectrally similar dyes
- Validated in multicolor STORM and 3D super-resolution microscopy (see p. 21)
- Compatible with LI-COR® Odyssey®

CF®680R Features

- Unrivaled photostability among spectrally similar dyes
- Compatible with STED, STORM, single molecule spectroscopy, and 2-photon microscopy (see p. 21)
- Molecular weight compatible with nucleic acid labeling
- Compatible with LI-COR® Odyssey®

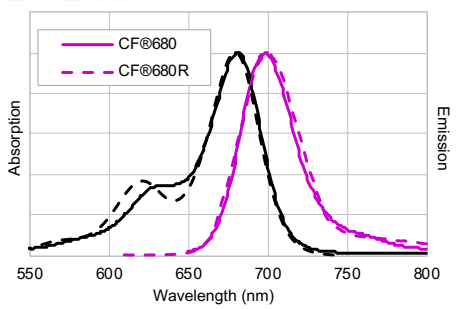


Figure 1. Absorption and emission spectra of CF®680 and CF®680R goat anti-mouse conjugates in PBS.

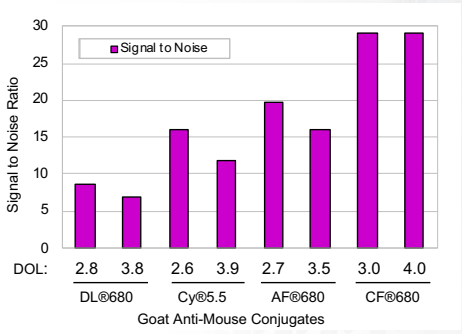


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-human CD3 antibody or isotype control followed by goat anti-mouse secondary antibody conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel. Bars represent the signal-to-noise ratio of CD3-positive fluorescence to isotype control.

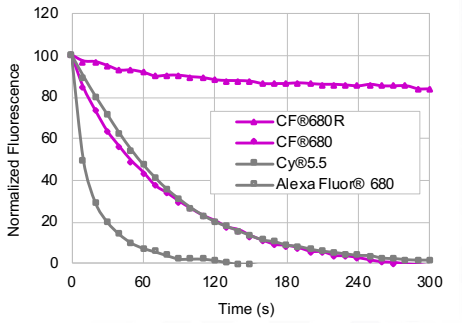


Figure 3. Relative photostability of far-red dye conjugates. Jurkat cells were stained with mouse anti-CD3 followed by the indicated goat anti-mouse IgG conjugates. Cells were continuously exposed to mercury arc lamp excitation with a Cy®5 filter set. Images were captured every 10 seconds for 5 minutes and fluorescence intensity was normalized to time 0.

CF®700 to CF®820

Unrivaled near-infrared dyes

Technical Summary

Dye	Ex/Em (nm)	Extinction coefficient	MW	Laser line	Spectrally similar to
CF®700	695/720	240,000	~2315	633-685 nm	Alexa Fluor® 700, DyLight® 700
CF®750	755/777	250,000	~3009	633-685 nm	Alexa Fluor® 750, Cy®7, DyLight® 750
CF®770	770/797	220,000	~3138	785 nm	DyLight® 800, IRDye® 800CW
CF®790	784/806	210,000	~3267	785 nm	Alexa Fluor® 790
CF®800	797/816	210,000	~3334	785 nm	Indocyanine Green
CF®820	822/835	253,000	~2553	785 nm	Unique near-IR dye

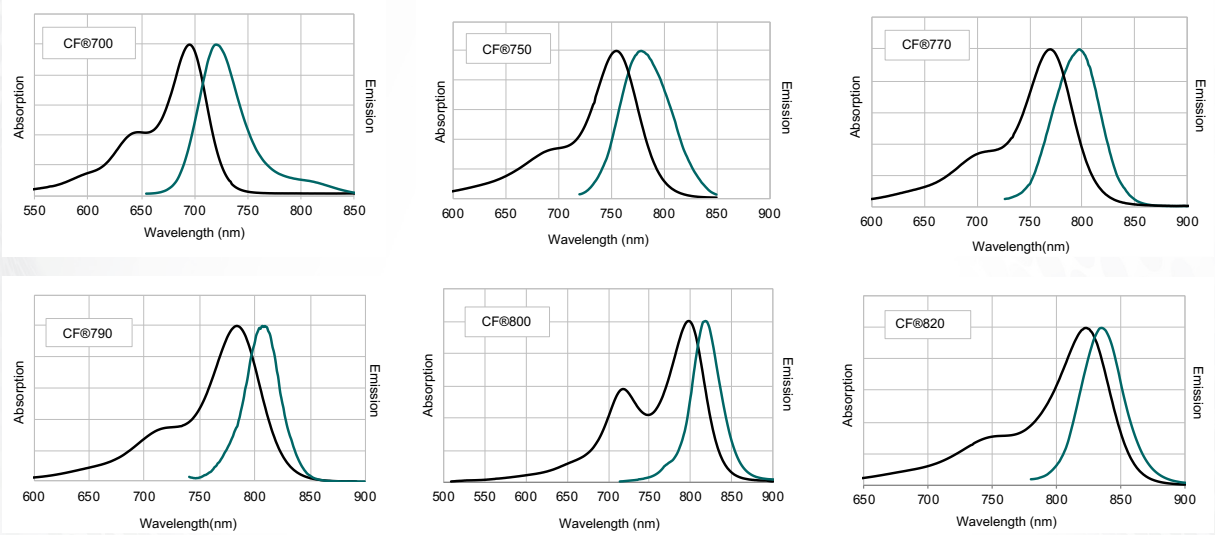


Figure 1. Absorption and emission spectra of near-IR CF® dye goat anti-mouse conjugates in PBS.

Features

- Exceptionally bright and stable
- Patented PEGDye™ technology for superior performance (see p. 3)
- Ideal for *in vivo* imaging
- Compatible with LI-COR® Odyssey®
- Superior signal-to-noise for conjugates
- CF®750 validated in STORM and photoacoustic imaging (see p. 21)

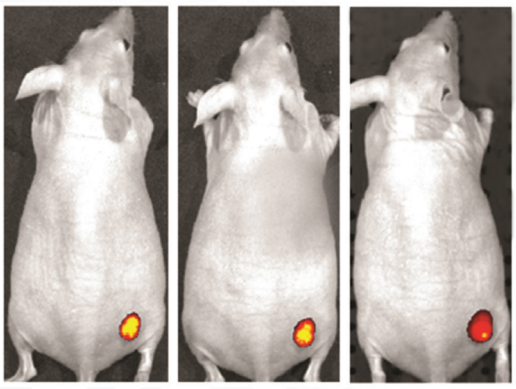


Figure 2. Tumors in mice were imaged using an IVIS® imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin conjugated to CF®750. Images courtesy of Caliper Life Sciences.

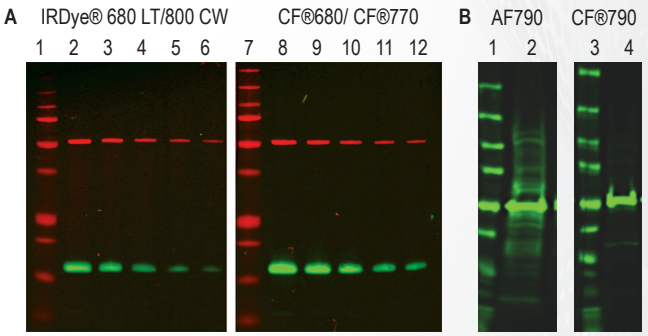


Figure 3. Near-IR western blotting with CF® dyes compared to spectrally similar dyes. A. Two-fold dilutions of HeLa cell lysate containing from 2 ug to 0.125 ug total protein per lane were separated by SDS-PAGE, transferred to a PVDF membrane, and probed with mouse anti-tubulin and rabbit anti-COXIV antibodies. Secondary detection was performed with either IRDye® 680LT goat anti-mouse (red) and IRDye® 800CW goat anti-rabbit (green) (LI-COR®; lanes 1-6) or CF®680 goat anti-mouse (red) and CF®770 goat anti-rabbit (green) (lanes 7-12) at the same final concentrations. Membranes were scanned using an Odyssey® infrared imaging system. Quantitation showed approximately 1.5- to 2-fold higher fluorescence intensity of CF® dye secondary antibodies compared to IRDye® secondary antibodies. B. Western blots of HeLa cell lysate (lanes 2 and 4) were probed with mouse anti-tubulin antibody followed by goat anti-mouse conjugated to Alexa Fluor® 790 (AF790, left) or CF®790 (right). CF®790 does not introduce excessive negative charge to antibody conjugates, which can increase non-specific binding.

CF® Dyes for Super-Resolution Imaging

Recent publications comparing synthetic dyes for super-resolution imaging have shown CF® dyes give the best performance for multiple methods. The superior brightness, photostability, and photochemical switching properties of certain CF® dyes are ideal for 3-D SIM, 3-D STORM, and other super-resolution and single molecule imaging techniques. Biotium's CF®405M has been found to be the brightest and most photostable short wavelength fluorescent dye for SIM. Six CF® dyes spanning the visible red, far-red, and near-infrared spectra have been validated for STORM; including three color imaging with CF®568, CF®647, and CF®680.[1] See page 21 for a list of CF® dyes validated in super-resolution and 2-photon imaging and other super-resolution techniques.

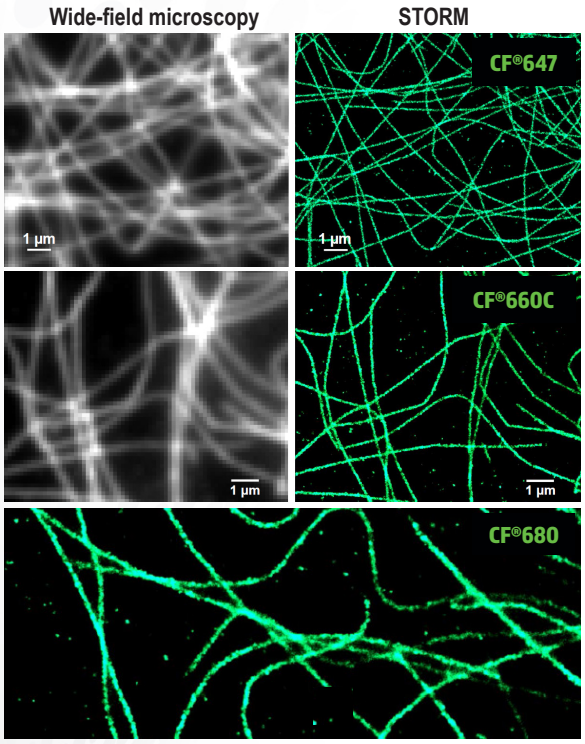


Figure 1. Comparison of conventional wide-field microscopy (left) with STORM (right) using CF® dye conjugates. Fixed cells were stained with mouse anti-tubulin antibody followed by CF® dye conjugated anti-mouse secondary antibody (top row: CF®647, middle row: CF®660C, bottom row: CF®680). For STORM, samples were sealed in buffer that contained 5% (w/v) glucose, 100 mM cysteamine, 0.8 mg/mL glucose oxidase, and 40 µg/mL catalase, in Tris-HCl (pH 7.5). Samples were imaged on a Nikon Ti-Eclipse w/ PFS microscope with a CFI Plan Apo Lambda 100x oil objective. Dye molecules were photoswitched and imaged using a 647 nm laser; a 405 nm laser was used to assist dye reactivation to the emitting state. Emission was collected with an Andor iXon Ultra 897 EMCCD camera for a total of 100,000 frames per image at a frame rate of 110 Hz. Dr. Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

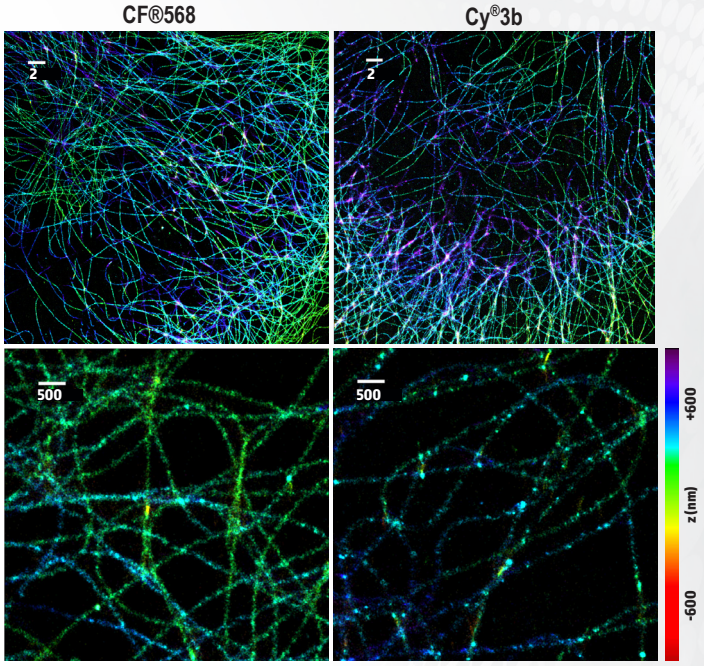


Figure 2. CF®568 (left) produces better images than Cy®3b (right) in 3-D STORM microscopy. Fixed cells were stained with mouse anti-tubulin antibody followed by dye-conjugated anti-mouse secondary antibodies. See Figure 1 for imaging conditions. Dye molecules were photoswitched and imaged using a 560 nm laser; a 405 nm laser was used to assist dye reactivation to the emitting state. Dr. Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

See page 4 for dye applications at a glance.

Single-Label Secondary Antibody Conjugates for STORM

Secondary antibodies with a low degree of labeling (DOL, or number of dye molecules per antibody) have been reported to be optimal for STORM.[2] We offer single-label secondary antibody conjugates of our STORM-compatible dyes with an average DOL of one dye per antibody.

See page 26-29 for our full selection of antibody conjugates.

Secondary Antibodies, Single Label for STORM

1 mg/mL in PBS. 50% glycerol, 2 mg/ml BSA, 0.05% sodium azide; unit sizes: 0.5 mL, 50 uL

Conjugate	Donkey anti-goat	Donkey anti-guinea pig	Donkey anti-mouse (min x rat)	Donkey anti-rabbit	Goat anti-mouse	Goat anti-rabbit
Min x react	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm	Bv, Ch, Gt, Hs, Hu, Ms, Rb, Sh, SHm	Bv, Ch, Gt, GP, Hs, Hu, Rb, Rt, Sh, SHm	Bv, Ch, Gt, GP, Hs, Hu, Ms, Sh, SHm	Bv, Hs, Hu, Rb, Sw	Hu, Ms, Rt
CF®535ST			20823	20824	20821	20822
CF®568	20836	20838	20802	20803	20800	20801
CF®594ST			20806	20807	20804	20805
CF®647	20829	20837	20810	20811	20808	20809
CF®660C			20815	20816	20812	20813
CF®680			20819	20820	20817	20818
CF®750			20827	20828	20825	20826

Bv: bovine; Ch: chicken; Gt: goat; GP: guinea pig; Hs: horse; Hu: human; Ms: mouse; Rb: rabbit; Sh: sheep; SHm: Syrian hamster; Sw: swine; Rt: rat

1. Lehmann et al. (2016) J Biophotonics 9, 161.
2. Bittel et al. (2015) Proc SPIE 9331, 933110M

CF® Dyes for Super-Resolution Imaging

& Other specialized applications

CF® dye	Abs/Em maxima	Extinction coefficient	Application	References
CF®405S	404/431 nm	33,000	SIM	Demmerle, J. et al. (2017). Nature Protocols 12, 988–1010. Essig, K. et al. (2017). Immunity https://doi.org/10.1016/j.immuni.2017.11.008
CF®405M	408/452 nm	41,000	SIM, STED	Kraus, F. et al. (2017). Nat Protoc 12, 1011-1028. doi:nprot.2017.020 (SIM) Markaki, Y. et al. (2013). Methods Mol Biol 950, 43-64. (SIM) Miron, E. et. al. (2016). In: Mark C. Leake (ed.), Methods in Molecular Biology, vol. 1431, 127-140. (SIM) Ohgomori, T. et al. (2017). Eur J Neurosci 46, 2001-2014. doi:10.1111/ejn.13650 (SIM) Zhang, R. et al. (2019). bioRxiv doi: https://doi.org/10.1101/586461 (STED)
CF®488A	490/515 nm	70,000	STED, STORM, TIRF, 2-Photon	Angelov, B. & Angelova, A. (2017). Nanoscale 9, 9797-9804. doi:10.1039/c7nr03454g (STED) Mercier, L. et al. (2016). Intravital 5, e1168553 (2-Photon) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200. (TIRF) Collaborator communication (STORM); contact tech support through our website for more information.
CF®535ST	535/568 nm	95,000	STORM	Collaborator communication; contact tech support through our website for more information.
CF®555	555/565 nm	150,000	Multicolor STORM	Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119
CF®568	562/583 nm	100,000	Multicolor STORM, SIM, TIRF	Gong, Y.-N. et al. (2017). Cell Cycle, 1-13. doi:10.1080/15384101.2017.1371889 (STORM) Gorur, A. et al. (2017). J Cell Biol 216, 1745-1759. doi:10.1083/jcb.201702135 (STORM) Heller, J. (2017). OM&P 3, 48-58, doi:doi:10.20388/omp2017.002.0045 (STORM) Jorgans, D.M. et al. (2017). J Cell Sci 2017 130: 177-189. doi: 10.1242/jcs.190967 (STORM) Karanasios, E. et al. (2016). Nat Commun 7: 12420. DOI: 10.1038/ncomms12420 (STORM) Kraus, F. et al. (2017). Nat Protoc 12, 1011-1028. doi:nprot.2017.020 (SIM) Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119 (STORM) Lim, A. et al. (2017). Mol Biol Cell doi:mbc.E16-12-0820 (SIM) Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8 (STORM) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200.(TIRF) Zhang, M. et al. (2015). eLife 2015;10.7554/eLife.11205 (STORM)
CF®594	593/614 nm	115,000	2-Photon	Wagner, M.C. (2016). Am J Physiol Renal Physiol310: F1089–F1102. (2-Photon)
CF®594ST	593/614 nm	115,000	STORM	Collaborator communication; contact tech support through our website for more information.
CF®633	630/650 nm	100,000	FIONA, gSHRImP, Single molecule tracking, TIRF	Bosch, P. J. et al. (2014). Biophys J 107, 803-814. (TIRF) Huang, T. et al. (2018). Biophysical Journal 114, 301–310. (Single Molecule Tracking) Kim, H. J., and Selvin, P. R. (2013). SpringerReference Encyclopedia of Biophysics. (FIONA) Simonson, P. D. et al. (2011). Nano Lett 11, 5090-5096. DOI:10.1021/nl203560r (gSHRImP) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200. (TIRF) Zhang, R. et al. (2017). eLife 2017;6:e30959. (TIRF)
CF®640R	642/662 nm	105,000	FLImP, SIM, TIRF	Bosch, P. J. et al. (2014). Biophys J 107, 803-814. (TIRF) Loh, L. N. (2017). MBio 8, doi:mBio.02030-16 (SIM) Martin-Fernandez, M. L. et al. (2013). J Microsc 252, 16-22. (TIRF) Needham, S.R. et al. (2015). Biochem Soc Trans 43, 309–314. (FLImP) Needham, S.R. et al. (2016). Nat Commun 7, 13307. doi:ncomms13307 (FLImP) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200. (TIRF) Zanetti-Domingues, L.C. et al. (2015). Prog Biophys Mol Biol. doi:S0079-6107(15)00047-4 (FLImP)
CF®647	650/665 nm	240,000	Multicolor STORM	Gong, Y.-N. et al. (2017). Cell Cycle, 1-13. doi:10.1080/15384101.2017.1371889 Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119 Olivier, N. et al. (2013). Biomed Opt Express 4, 885-899. Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8
CF®660C	667/685 nm	200,000	Multicolor STORM	Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8 Zhang, Z., et al. (2015). Nature Methods doi:10.1038/nmeth.3528.
CF®680	681/698 nm	210,000	Dual-Color 3D SMLM, Multicolor STORM	Früh, S.M. et al. (2015). Nature Communications 6, 7275. (STORM) Glebov, O.O. et al. (2017). Cell Rep 18, 2715-2728. doi:S2211-1247(17)30279-6 (STORM) Gorur, A. et al. (2017). J Cell Biol 216, 1745-1759. doi:10.1083/jcb.201702135 (STORM) Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119 (STORM) Platonova, E. et al. (2015). ACS Chem. Biol.10(6),1411–1416. (STORM) Platonova, E. et al. (2015). Methods doi: http://dx.doi.org/10.1016/j.ymeth.2015.06.018. (STORM) Salvador-Gallego, R. et al. (2016). EMBO J. DOI 10.15252/embj.201593384. (STORM) Shrestha, R. L. et al. (2017). Nat Commun 8, 150. doi:10.1038/s41467-017-00209-z (STORM) Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8 (SMLM) Winterflood, C.M. et al. (2015). Biophys J. 109, 3–6. (SMLM) Zhang, Z., et al. (2015). Nature Methods doi:10.1038/nmeth.3528. (STORM)

FIONA: Fluorescence Imaging with One Nanometer Accuracy; FLImP: Fluorophore localization imaging with photobleaching; SHRImP: Single-molecule High-Resolution Imaging with Photobleaching; SIM: Structured Illumination Microscopy; STED: Stimulated Emission Depletion; STORM: Stochastical Optical Reconstruction Microscopy; TIRF: Total Internal Reflection Fluorescence

Mix-n-Stain™ Antibody Labeling Kits

Mix-n-Stain™ CF® dye & Hapten Antibody Labeling Kits

- Labeling in 30 minutes with minimal hands-on time & no purification
- Covalent conjugation, suitable for multiplex staining
- Choice of small-scale labeling sizes to conserve precious antibodies
- Reaction tolerates common antibody buffers & stabilizers
- Modified protocol for antibodies with excess BSA/gelatin or ascites

Antibody Labeling Made Simple

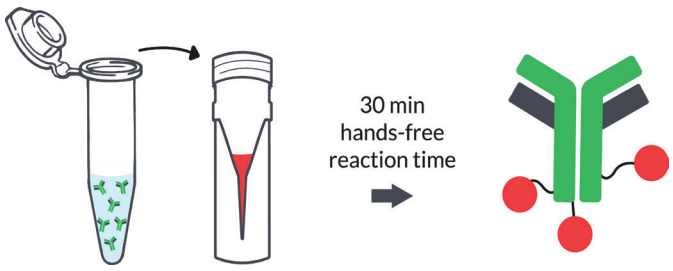


Figure 1. Mix-n-Stain™ CF® dye labeling protocol. Simply mix your antibody with the reaction buffer and pre-measured dye, and incubate 30 minutes for a ready-to-use conjugate covalently labeled with one of our bright & photostable CF® dyes, biotin, or other label.

Mix-n-Stain™ Labeled Antibodies Perform Better Than Lightning-Link® Labeled Antibodies

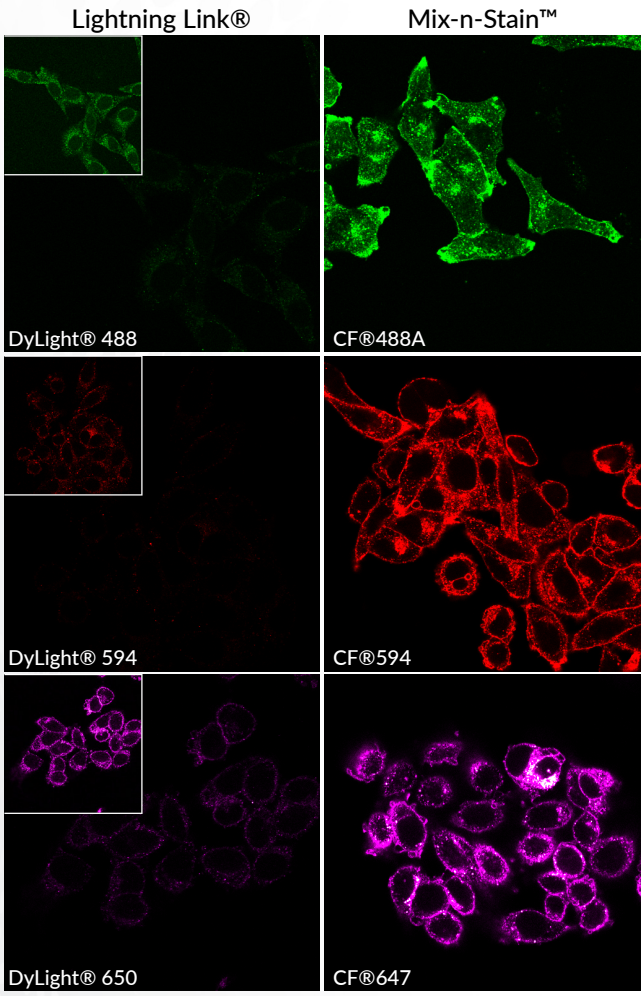


Figure 2. Mouse anti-transferrin receptor antibody from BD Biosciences (endosome and plasma membrane marker) was labeled using Lightning-Link® Rapid Conjugation Kits with the indicated DyLight® dyes (left) or Mix-n-Stain™ CF® dye Antibody Labeling Kits (right). CF® dye conjugates shows brighter signal and more specific staining compared to the spectrally similar DyLight® conjugates using the same laser and gain settings. The insets show the DyLight® conjugates imaged with higher gain settings to demonstrate the presence of cells in the field of view.

Mix-n-Stain™ CF® Dye or Hapten Antibody Labeling Kits

Dye or Label	Ex/Em (nm)	1 x 5-20 ug labeling	1 x 20-50 ug labeling	1 x 50-100 ug labeling	Mix-n-Stain™ Maxi 1 mg labeling
CF®350	347/448	92270	92250	92230	92420
CF®405S	404/431	92271	92251	92231	92421
CF®405M	408/452	92272	92252	92232	92404
CF®405L	395/495	92303	92304	92305	
CF®430	426/498	92316	92317	92318	
CF®440	440/515	92319	92320	92321	
CF®450	405/460	92322	92323	92324	
CF®488A	490/515	92273	92253	92233	92405
CF®514	516/548	92331	92332	92333	
CF®532	527/558	92289	92290	92291	
CF®543	541/560	92287	92267	92247	
CF®555	555/565	92274	92254	92234	92406
CF®568	562/583	92275	92255	92235	92407
CF®570	568/591	92334	92335	92336	
CF®583	586/609	92337	92338	92339	
CF®594	593/614	92276	92256	92236	92408
CF®633	630/650	92277	92257	92237	92409
CF®640R	642/662	92278	92258	92245	
CF®647	650/665	92279	92259	92238	92410
CF®660C	667/685	92280	92260	92239	
CF®660R	663/682	92281	92261	92243	
CF®680	681/698	92282	92262	92240	92422
CF®680R	680/701	92283	92263	92246	
CF®700	695/720	92425	92426	92427	
CF®750	755/777	92284	92264	92241	92423
CF®770	770/797	92285	92265	92242	92424
CF®790	784/806	92288	92268	92248	
CF®800	797/816	92428	92429	92430	
CF®820	822/835	92431	92432	92433	
FITC	494/519	92294	92295	92296	92411
Cyanine 555[1]	555/565	92412	92413	92414	92415
Cyanine 647[2]	650/665	92416	92417	92418	92419
Biotin	N/A	92286	92266	92244	
Digoxigenin	N/A	92328	92329	92330	
DNP	N/A	92325	92326	92327	

[1] Structurally identical to Cy®3
[2] Structurally identical to Cy®5

Antibody, Protein, and Ligand Labeling Kits

Mix-n-Stain™ Enzyme or Fluorescent Protein Antibody Labeling Kits

- Easy conjugation in just a few hours with no special equipment required
- Choose AP, HRP, or GOx enzyme conjugation
- Easy labeling with R-PE, APC, PerCP, or tandem dyes for flow cytometry

Mix-n-Stain™ Small Ligand Labeling Kits

- For labeling small molecules with primary amines
- Label SNAP-Tag®, CLIP-Tag™, HALO-Tag®, or TMP-tag ligands
- 30 minute labeling with minimal hands-on time and no purification
- Choose from 10 CF® dye colors for surface targets, or 5 CF® dye colors for intracellular targets

Mix-n-Stain™ Enzyme Antibody Labeling Kits

Conjugation	1 x 10-20 ug labeling	1 x 25-50 ug labeling	1 x 50-100 ug labeling	1 x 1 mg labeling
Horseradish peroxidase (HRP)	92300	92301	92302	92437
Alkaline phosphatase (AP)		92314	92315	
Glucose oxidase (GOx)		92312	92313	

Mix-n-Stain™ Fluorescent Protein Antibody Labeling Kits

Conjugation	Ex/Em (nm)	1 x 25-50 ug labeling	1 x 50-100 ug labeling	1 x 1 mg labeling
R-PE	496,564/578	92298	92299	
R-PE-CF®647T	496/665	92340	92341	92346
R-PE-CF®583R	496/609	92442	92443	
APC	650/660	92306	92307	
Per-CP	482/678	92308	92309	
APC-CF®750T	650/780	92310	92311	

CF® Dye SE and VivoBrite™ Protein Labeling Kits

- Everything you need to label and purify 3 x 1 mg antibody
- VivoBrite™ kits feature superior near-IR CF® dyes for *in vivo* imaging, and 0.2 um sterile mini-syringe filters

Mix-n-Stain™ Small Ligand Labeling Kits

Dye	Ex/Em (nm)	Cell surface targets	Intracellular targets
CF®405M	408/452	92362	
CF®408	408/450		92356
CF®488A	490/515	92350	
CF®500	500/510		92357
CF®540	540/570		92358
CF®555	555/565		92364
CF®568	562/583	92351	
CF®594	593/614	92352	
CF®633	630/650	92353	
CF®640R	642/662	92354	
CF®647	650/665	92359	
CF®650	650/670		92363
CF®660C	667/685	92360	
CF®680	681/698	92361	
CF®680R	680/701	92355	

CF® Dye or Biotin SE Protein Labeling Kits

Dye or Label	Ex/Em (nm)	Cat. No.
CF®350	347/448	92210
CF®405S	404/431	92211
CF®405M	408/452	92212
CF®405L	395/495	92228
CF®488A	490/515	92213
CF®532	527/558	92208
CF®543	541/560	92209
CF®555	555/565	92214
CF®568	562/583	92215
CF®594	593/614	92216
CF®633	630/650	92217
CF®640R	642/662	92225
CF®647	650/665	92218
CF®660C	667/685	92219
CF®660R	663/682	92223
CF®680	681/698	92220
CF®680R	680/701	92226
CF®750	755/777	92221
CF®770	770/797	92222
Biotin	N/A	92224

VivoBrite™ Antibody Labeling Kits for NIR Small Animal *In Vivo* Imaging

Dye	Ex/Em (nm)	Cat. No.
CF®680	681/698	92160
CF®750	755/777	92161
CF®770	770/797	92162
CF®790	784/806	92163

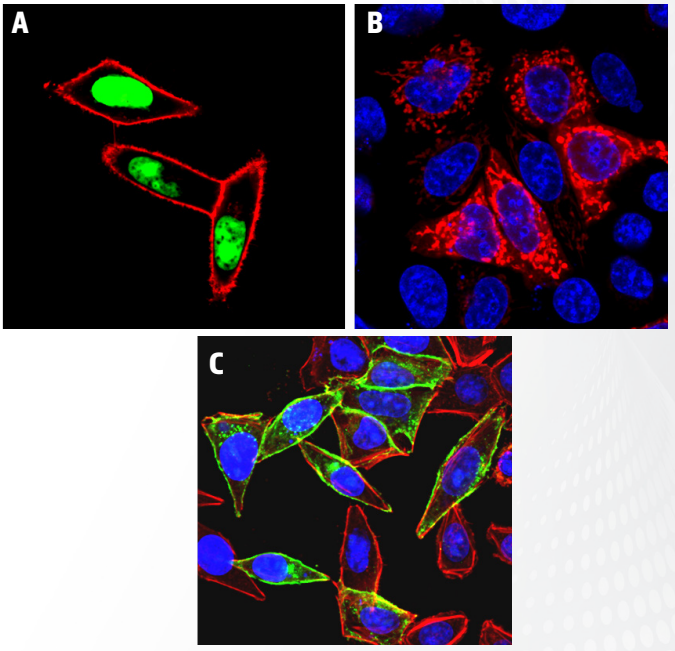


Figure 1. Versatility of Mix-n-Stain™-labeled ligands for multicolor live cell imaging. (A) CF®500-labeled CLIP-Tag™ ligand was used to detect nuclear protein H2B (green), and CF®568-labeled SNAP-Tag® ligand was used to detect cell surface protein ADRβ2. (B) CF®540-labeled CLIP-Tag™ ligand was used to detect mitochondrial protein Cox8A in living cells (red); nuclei were stained with Hoechst 33342 (blue). (C) Three color imaging in fixed cells. CF®488A-labeled CLIP-Tag™ ligand was used to stain cell surface protein NK1R (green). Cells were then fixed and stained with CF®633 phalloidin (red) and mounted with EverBrite™ mounting medium with DAPI (blue).

CF® Dye: Reactive Dyes

A wide selection of colors and functional groups for dye conjugation

Reactive group	Alkyne	Amine	Aminooxy	Azide	BCN	Hydrazide	Maleimide	MTS	Picolyl azide	Succinimidyl Ester (SE)	Tyramide[2]
Reacts with	Azides, picolyl azides	Activated carboxylic acids	Aldehydes & ketones	Alkynes, BCN (Cu-catalyzed)	Azides (Cu-free)	Polar tracer[1]	Thiols	Thiols	Alkynes (low [Cu])	Primary amines; lysine residues	HRP substrate
Size	0.5 mg	1 mg	1 mg	0.5 mg	0.5 mg	1 mg	1 umol	1 mg	0.5 mg	1 umol	0.5 mg
CF®350		92035	92050			92151	92020			92109	92170
CF®405S		92036	92055		92113	92183	92030			92110	92197
CF®405M	92093		92056	92092	92114		92021			92111	96057
CF®405L							92046			92112	92198
CF®430						96063	92118			92117	96053
CF®440					96070[3]	96064	92124			92123	
CF®450							96012			96011	
CF®488A	92086	92037	92051	92080	92075	92152	92022	92097	92187	92120	92171
CF®503R					96026[3]		96079			96078	
CF®514										92103	92199
CF®532				92180			92045			92104	96066
CF®543				92181			92044	92098		92105	92172
CF®550R	92087	92038		92081		92153	96704			96073	96077
CF®568	92088	92039	92057	92082	92076	92154	92024		92188	92131	92173
CF®570							96015			96014	
CF®583							96017			96016	
CF®594	92089	92040	92052	92083	92077	92158	92025	92099	92189	92132	92174
CF®620R							92033			92106	92194
CF®633		92041	92053			92156	92026			92133	
CF®640R	92091	92043	92058	92085	92078	92157	92034	92096	92190	92108	92175
CF®647	92090	92042		92084		92136	92027		92191	92135	96022
CF®650					96027[3]						
CF®660C	92095			92094			92028		96001	92137	
CF®660R	96004	96010	92059	92182		96024	92031		96002	92134	92195
CF®680	96005			92119			92029		96003	92139	
CF®680R	96006		92054	96000	92079	96025	92032		96007	92107	92196
CF®700										96067	
CF®750		92102					96062			92142	96052
CF®770		92065				92192				92150	
CF®790										92155[4]	
CF®800							92128			92127[4]	
CF®820										96068[4]	

[1] For conjugation to aldehyde or ketone groups, we recommend using CF® dye aminooxy forms.
[2] See page 30 for Tyramide Amplification Kits and Ready-to-Use Tyramide Amplification Buffer.
[3] Membrane-permeant, compatible with intracellular copper-free reaction with azide.
[4] Size: 0.25 umol

Don't see what you're looking for?

We regularly add new CF® dye products to our catalog according to customer demand. Be sure to check our website for updates. If you are looking for a CF® dye product not listed in our catalog, please contact tech support through our website. We may be able to add it as a new product, or perform a custom synthesis for you.

Visit www.biotium.com to see our full selection of reactive biotin reagents, traditional reactive dyes, Cyanine Dyes (structurally equivalent to Cy® dyes), and sets of size- and charge-matched dyes for DIGE.

CF® Dye Bioconjugates

Bioconjugate Applications

Conjugate	Application
Annexin V	Phosphatidylserine probe; apoptotic cell surface marker Available in solution with azide, or lyophilized, azide-free for real-time imaging
a-Bungarotoxin (BTX)	Acetylcholine receptor probe; neuromuscular junction stain
Bovine serum albumin (BSA)	Fluid-phase endocytosis tracer; in vivo blood flow tracer
Cholera Toxin Subunit B	GM1 receptor probe; lipid raft, endocytic vesicle, neuronal tracing
Concanavalin A (Con A)	Lectin; binds α-D-mannosyl and α-D-glucosyl groups, stains yeast cell wall
Dextran amine, anionic	Fixable fluid-phase endocytosis tracer
Nucleotide conjugates	Fluorescent DNA or RNA probe synthesis; TUNEL apoptosis assay
Phalloidin	Filamentous actin probe
Peanut agglutinin (PNA)	Lectin; specific for terminal b-galactose
Streptavidin	Detection of biotinylated probes
Transferrin (human)	Recycling endosome tracer
Wheat germ agglutinin (WGA)	Lectin, binds N-acetyl-D-glucosamine and sialic acid; Fluorescent bacterial Gram stain, stains yeast bud scars

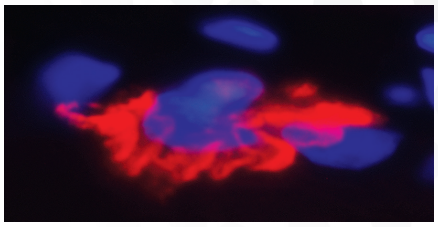


Figure 1. Frozen section of rat skeletal muscle stained with CF®633 a-bungarotoxin (magenta) to detect nicotinic acetylcholine receptors at the neuromuscular junction. Nuclei are stained with DAPI (blue).

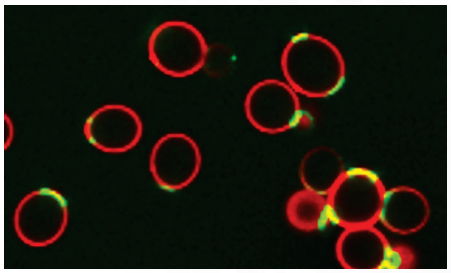


Figure 2. *S. cerevisiae* yeast stained with CF®488A WGA and CF®594 ConA. ConA (red) stains the cell wall, while WGA (green) preferentially stains bud scars.

CF® Dye Bioconjugates

Dye	Annexin V	Annexin V, azide-free	a- BTX	BSA	Cholera Toxin B	Con A	Dextran 3.5K	Dextran 10K	Dextran 40K	Dextran 70K	Dextran 150K	Dextran 250K	Phalloidin	PNA	Streptavidin	Transferrin	WGA
CF®350	29012	29012R-5ug				29015	80137						00049		29031		29021
CF®405S			00002			29075									29032		29027
CF®405M	29009	29009R-5ug				29074							00034		29033		29028
CF®405L															29056		
CF®430													00054		29065		
CF®440													00055		29066		
CF®450	29083	29083R-5ug															
CF®488A	29005	29005R-5ug	00005	20289	00070	29016		80110	80126	80117	80131	80134	00042	29060	29034	00081	29022
CF®514															29081		
CF®532					00074								00051		29030		29064
CF®543			00026		00075			80111					00043		29043	00082	
CF®555	29004	29004R-5ug	00018					80112					00040		29038		29076
CF®568	29010	29010R-5ug	00006		00071			80113					00044	29061	29035	00083	29077
CF®583R		29085R-5ug											00064				
CF®594	29011	29011R-5ug	00007	20290	00072	29017		80114					00045	29062	29036	00084	29023
CF®620R					00076												
CF®633	29008	29008R-5ug	00009		00077	29018							00046		29037		29024
CF®640R	29014	29014R-5ug	00004	20291	00073	29019		80115					00050	29063	29041	00085	29026
CF®647	29003	29003R-5ug											00041		29039		
CF®660C													00052				
CF®660R	29069	29069R-5ug			00078								00047		29040		
CF®680		29007		20292		29020		80118	80127	80129	80132	80135	00053				29029
CF®680R		29070	00003		00079			80116					00048		29072	00086	29025
CF®700		29082															
CF®750		29006				29080		80119	80128	80130	80133	80136				00087	
CF®770		29046				29058		80120	80122	80123	80124	80125					29059
CF®790		29047						80121									
CF®800		29078															

Visit www.biotium.com to see our selection of apoptosis staining kits, bacterial Gram stain kits, and phalloidin conjugates of biotin and traditional dyes.

Nucleotide Conjugates

Nucleotide	CF®405S	CF®405M	CF®488A	CF®532	CF®543	CF®555	CF®568	CF®594	CF®640R	CF®647	CF®660R	CF®680R
dCTP			40067	40057	40058	40027	40055	40056	40066	40028	40068	
ddCTP						40031						
UTP									40032			
dUTP	40004	40100	40008		40002		40005	40006	40007			40003

Visit www.biotium.com to see our CF® dye TUNEL staining kits, plus a selection of nucleotide conjugates of biotin, traditional dyes, and amino-allyl nucleotides.

Primary Antibody Conjugates

Features

- More than 1000 monoclonal antibodies
- Growing selection of recombinant monoclonal mAbs & monoclonal rabbit antibodies
- Validated in IHC and other applications
- Select mAbs verified as monospecific in HuProt™ human protein array
- Choice of 13 bright and photostable CF® dyes
- Also available with R-PE, APC, PerCP, HRP, AP, or biotin
- Matched isotype controls for mouse and rabbit monoclonal antibodies
- Purified antibodies available BSA-free, 1 mg/mL, and ready-to-use for Mix-n-Stain™ labeling or other conjugation

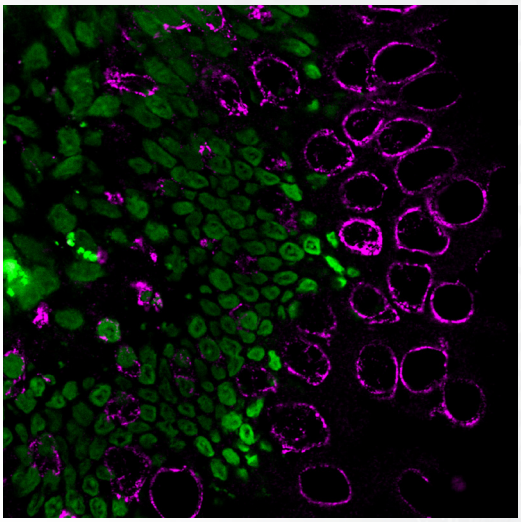


Figure 3. Immunofluorescence staining of rat jejunum with CF®488A mouse anti-Histone H1 (nuclei, green) and CF®647 mouse anti-Pan Cytokeratin (microfilaments, magenta).

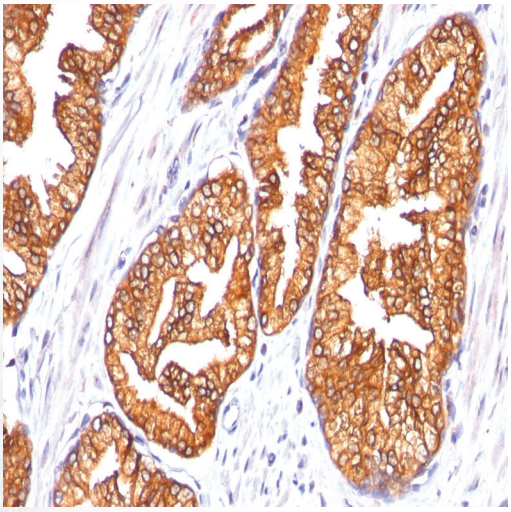


Figure 1. IHC staining of human prostate carcinoma with anti-ODC1 clone ODC1/485.

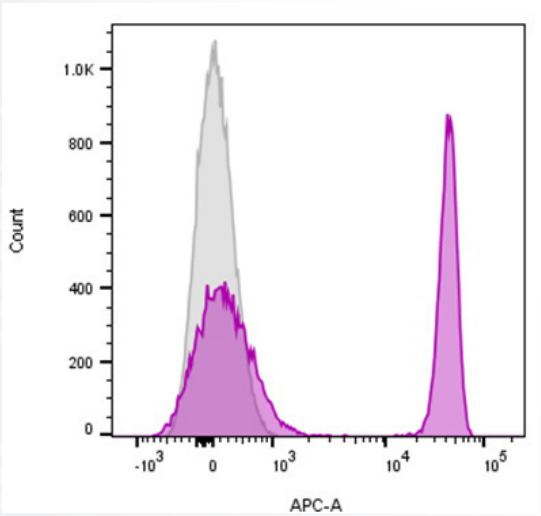


Figure 2. Surface staining of human PBMC with anti-CD4 (EDU-2) CF®640R conjugate (magenta) compared to unstained cells (gray).

Your Choice of Size and Format

Format	Concentration	Size
CF® dye conjugates (13 colors)	0.1 mg/mL	100 or 500 uL
Biotin, HRP, or AP conjugates	0.1 mg/mL	100 or 500 uL
R-PE, APC, or Per-CP conjugates	0.1 mg/mL	250 uL
Purified, with BSA	0.1 mg/mL	100 or 500 uL
Purified, BSA-free	1 mg/mL	50 uL

Your Choice of 13 Bright and Photostable CF® Dyes

CF® dye	Ex/Em (nm)	Features
CF®405S	404/431	• Better fit for the 450/50 flow cytometer channel than Alexa Fluor® 405
CF®405M	408/452	• More photostable than Pacific Blue™, with less green spill-over • Compatible with super-resolution imaging by SIM
CF®488A	490/515	• Less non-specific binding and spill-over than Alexa Fluor® 488 • Very photostable and pH-insensitive • Compatible with super-resolution imaging by TIRF
CF®543	541/560	• Brighter than Alexa Fluor® 546
CF®555	555/565	• Brighter than Cy®3 • Validated in multicolor super-resolution imaging by STORM
CF®568	562/583	• Optimized for the 568 nm line of the Ar-Kr mixed-gas • Brighter and more photostable than Alexa Fluor® 568 • Compatible with TIRF and multicolor STORM
CF®594	593/614	• Brighter than Texas Red® or Alexa Fluor® 594 • Extremely photostable
CF®640R	642/662	• Most photostable Cy®5-like dye with excellent brightness • Compatible with TIRF and FLImP super-resolution techniques
CF®647	650/665	• Brighter than Cy®5 • Compatible with super-resolution imaging by STORM
CF®660R	663/682	• Brighter than Alexa Fluor® 660, remarkably photostable
CF®680	681/698	• Brighter than Cy®5.5, Alexa Fluor® 680, or IRDye® 680LT • Validated in STORM and 3D super-resolution imaging • Compatible with LI-COR® Odyssey® System
CF®680R	680/701	• The most photostable 680 excitable dye • Compatible with LI-COR® Odyssey® System
CF®770	770/797	• Exceptionally bright and stable • Compatible with LI-COR® Odyssey® System • Replacement for DyLight® 800 or IRDye® 800CW

CF® Dye Anti-Tag and Secondary Antibody Conjugates

Anti-GFP, Anti-Hapten, and Anti-Epitope Tag Antibody Conjugates

In PBS, 50% glycerol, 2 mg/ml BSA, 0.05% sodium azide

Conjugate	Goat anti-GST 1 mg/mL 0.1 mL	Mouse monoclonal anti-biotin 2 mg/mL 50 uL or 0.25 mL	Mouse monoclonal anti-fluorescein 2 mg/mL 50 uL or 0.25 mL	Mouse monoclonal anti-GFP 1 mg/mL 0.1 mL	Mouse monoclonal anti-6X His tag 1 mg/mL 50 uL	Rabbit anti-HA tag 1 mg/mL 50 uL	Rabbit anti-RFP 1 mg/mL 0.1 mL	Rabbit anti-V5 tag 1 mg/mL 0.1 mL
CF®405S		20203						
CF®405M			20214					
CF®488A	20424	20204	20210	20215	20228	20238	20421	20440
CF®543							20476	20441
CF®568							20477	
CF®588				20480				20441
CF®594	20425	20205	20211	20216	20229	20239	20422	20442
CF®633		20206	20212	20217				
CF®640R	20426	20207	20213	20218	20237	20237	20423	20443
CF®647						20486		
CF®660R			20399	20481				
CF®680R				20482	20359		20478	
CF®750		20501		20220	20360			

Secondary Antibodies, Whole IgG (H+L), Not Cross-Adsorbed

2 mg/mL in PBS, 50% glycerol, 2 mg/ml BSA, 0.05% sodium azide, or preservative-free lyophilized form
Unit size: 0.5 mL, 50 uL, or 1 mg (lyophilized)

Conjugate	Chicken anti-goat	Chicken anti-mouse	Chicken anti-rabbit	Goat anti-guinea pig	Goat anti-llama	Goat anti-mouse	Goat anti-rabbit	Goat anti-swine	Llama anti-mouse	Llama anti-rabbit	Rabbit anti-chicken	Rabbit anti-goat	Rabbit anti-guinea pig
CF®350	20364	20331	20332	20198		20140	20141						
CF®405S					20844	20080	20082						
CF®405M						20180	20181						
CF®405L						20408	20409						
CF®488A	20225	20208	20209	20017	20845	20010	20012	20028	20454	20449	20079	20021	
CF®514						20386	20387						
CF®532						20365	20366						
CF®543	20333	20334	20335	20317	20846	20306	20309	20324			20312	20315	20336
CF®555				20036	20847	20030	20033	20236				20031	
CF®568	20337	20338	20339	20108	20848	20100	20102	20091	20455	20450		20107	
CF®594	20226	20221	20223	20118	20849	20110	20112	20160	20456	20451	20164	20117	
CF®633	20227	20222	20224	20129		20120	20122	20138			20165	20128	
CF®640R				20085	20850	20197	20202	20089	20457	20452		20090	
CF®647				20041	20851	20040	20043	20286	20458	20453		20049	
CF®660C					20852	20050	20053						
CF®660R					20853	20054	20055						
CF®680					20855							20068	20243
CF®750					20856	20070	20073						
CF®770													20244
CF®790						20378	20379						

Don't see what you're looking for?

We regularly add new CF® dye conjugates to our catalog according to customer demand. Be sure to check our website for updates. If you are looking for a CF® dye product not listed in our catalog, please contact tech support through our website and let us know. We may be able to add it as a new product, or perform a custom conjugation for you.

Visit www.biotium.com to see our full selection of secondary antibodies, including conjugates of biotin, HRP, R-PE, and APC.

CF® Dye Secondary Antibody Conjugates

Highly cross-adsorbed for multiple labeling

Drop-n-Stain™ Secondary Antibodies, Whole IgG (H+L), Highly Cross-Adsorbed

5 mL solution in convenient dropper bottle format for quick and easy immunofluorescence staining

Conjugate	Donkey anti-mouse (min x rat)	Donkey anti-rabbit	Goat anti-mouse	Goat anti-rabbit
Min x react	Bv, Ch, Gt, GP, Hs, Hu, Rt, Rb, Sh, SHm	Bv, Ch, Gt, GP, Hs, Hu, Ms, Rt, Sh, SHm	Bv, Hs, Hu, Rb,Sw	Hu, Ms, Rt
CF®488A	20952	20950	20956	20954
CF®543	20967	20966	20969	20968
CF®594	20953	20951	20957	20955
CF®640R	20963	20962	20965	20964

Bv: bovine; Ch: chicken; Gt: goat; GP: guinea pig; Hs: horse; Hu: human; Ms: mouse; Rb: rabbit; Sh: sheep; SHm: Syrian hamster; Sw: swine; Rt: rat

Secondary Antibodies, Whole IgG (H+L), Highly Cross-Adsorbed

2 mg/mL in PBS. 50% glycerol, 2 mg/ml BSA, 0.05% sodium azide, or preservative-free lyophilized form

CF®350 through CF®660R unit sizes: 0.5 mL, 50 uL, or 1 mg (lyophilized); near-IR conjugates available in 0.25 mL or 50 uL sizes

Conjugate	Bovine anti-goat	Donkey anti-chicken	Donkey anti-goat	Donkey anti-guinea pig	Donkey anti-human	Donkey anti-mouse (min x rat)	Donkey anti-rabbit	Donkey anti-rat	Donkey anti-sheep
Min x react	Bv, Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm	Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm	Bv, Ch, Gt, Hs, Hu, Ms, Rb, Sh, SHm	Bv, Ch, GP, Gt, Hs, Ms, Rb, Rt, Sh, SHm	Bv, Ch, Gt, GP, Hs, Hu, Rb, Rt, Sh, SHm	Bv, Ch, Gt, GP, Hs, Hu, Ms, Sh, SHm	Bv, Ch, GP, Gt, Hs, Hu, Ms, Rb, Sh, SHm	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm
CF®350		20275	20142			20350	20351	20361	20148
CF®405S			20416	20356			20420	20419	
CF®405M			20398	20376					
CF®430						20461	20462		
CF®488A	20293	20166	20016	20169	20074	20014	20015	20027	20024
CF@514						20483			
CF®543	20313	20310	20314	20316	20318	20305	20308	20320	20322
CF®555			20039	20276		20037	20038		20234
CF®568	20294		20106	20377		20105	20098	20092	20095
CF®594	20295	20167	20116	20170	20075	20115	20152	20159	20156
CF®633	20296	20168	20127	20171	20076	20124	20125	20137	20134
CF®640R	20297		20179			20177	20178	20199	20083
CF®647			20048			20046	20047	20843	20284
CF®660C			20051	20372					
CF®660R			20391			20388	20389	20390	
CF®680			20060	20241	20278		20418	20417	20062
CF®680R			20196			20194	20195		
CF®750			20362				20298	20857	
CF®770			20277	20242			20484		
CF®790			20345		20279	20363	20344		
CF@800			20834			20835			

Bv: bovine; Ch: chicken; Gt: goat; GP: guinea pig; Hs: horse; Hu: human; Ms: mouse; Rb: rabbit; Sh: sheep; SHm: Syrian hamster; Sw: swine; Rt: rat

Don't see what you're looking for?

We regularly add new CF® dye conjugates to our catalog according to customer demand. Be sure to check our website for updates. If you are looking for a CF® dye product not listed in our catalog, please contact tech support through our website and let us know. We may be able to add it as a new product, or perform a custom conjugation for you.

Visit www.biotium.com to see our full selection of secondary antibodies, including conjugates of biotin, HRP, R-PE, and APC.

CF® Dye Secondary Antibody Conjugates

Highly cross-adsorbed, F(ab')$_2$ fragments, and isotype-specific secondary antibodies

Secondary Antibodies, Whole IgG (H+L) , Highly Cross-Adsorbed (continued from p. 28)

2 mg/mL in PBS. 50% glycerol, 2 mg/ml BSA, 0.05% sodium azide, or preservative-free lyophilized form
CF®350 through CF®660R unit sizes: 0.5 mL, 50 uL, or 1 mg (lyophilized); CF®680 through CF®790 available in 0.25 mL or 50 uL sizes

Conjugate	Goat anti-chicken	Goat anti-guinea pig	Goat anti-human	Goat anti-mouse	Goat anti-mouse (min x rat)	Goat anti-rabbit	Goat anti-rat	Rabbit anti-human	Rabbit anti-mouse	Rabbit anti-rat	Rabbit anti-sheep
Min x react	Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm	Bv, Ck, Gt, Hs, Hu, Ms, Rb, Rt, SHm, Shp	Bv, Hs, Ms	Bv, Hs, Hu, Rb, Sw	Bv, Ch, Gt, GP Hs Hu Rb Rt, Sh, SHm	Hu, Ms, Rt	Bv, Hs, Hu, Rb	Ms	Hu	Hu	Hu
CF®350				20143		20144	20147		20149		
CF®405S		20488			20830						
CF®405M	20375	20487		20182	20340	20373	20374				
CF®430				20459		20460					
CF®488A	20020	20489	20022	20018	20302	20019	20023	20071	20026	20025	20172
CF®532				20468		20469					
CF®543	20311	20492	20319	20299	20328	20300	20321		20307		20323
CF®555	20034	20491	20320	20231		20232	20233		20235		
CF®568	20104	20492	20097	20101	20301	20103	20096		20093	20094	
CF®594	20114	20493	20154	20111	20303	20113	20155	20072	20158	20157	20173
CF®633	20126		20132	20121	20341	20123	20133	20066	20136	20135	20174
CF®640R	20084	20494	20081	20175	20304	20176	20088		20200	20201	
CF®647	20044	20495	20280	20281		20282	20283		20285		
CF®660C	20371	20497		20052	20368	20369	20370				
CF®660R		20496									
CF®680		20499	20287	20065		20067	20069		20061		
CF®680R		20498		20192		20193					
CF®750				20463							
CF®770		20500	20288	20077		20078	20383				
CF®790				20342		20343					

Bv: bovine; Ch: chicken; Gt: goat; GP: guinea pig; Hs: horse; Hu: human; Ms: mouse; Rb: rabbit; Sh: sheep; SHm: Syrian hamster; Sw: swine; Rt: rat

Secondary Antibodies, F(ab')$_2$ Fragments

2 mg/mL, unit size: 0.25 mL or 50 uL

Conjugate	Goat anti-mouse, F(ab')$_2$	Goat anti-rabbit, F(ab')$_2$
CF®350	20145	20146
CF®488A	20011	20013
CF®543	20329	20330
CF®555	20032	20035
CF®568	20109	20099
CF®594	20119	20153
CF®633	20130	20131
CF®640R	20086	20087
CF®647	20042	20045
CF®680	20063	20064

Goat Anti-Mouse Isotype-Specific Antibodies

2 mg/mL, unit size: 0.25 mL or 50 uL

Conjugate	Goat anti-mouse IgG1	Goat anti-mouse IgG2a	Goat anti-mouse IgG2b	Goat anti-mouse IgM
Min x react	Bv, Hu, Rb	Bv, Hu, Rb	Bv, Hu, Rb	Bv, Hu, Rb
CF®350	20245	20255	20265	
CF®405S	20380	20381	20382	
CF®488A	20246	20256	20266	20840
CF®543	20325	20356	20326	
CF®555	20247	20257	20267	20485
CF®568	20248	20258	20268	
CF®594	20249	20259	20269	
CF®633	20250	20260	20270	
CF®640R	20251	20261	20271	
CF®647	20252	20262	20272	
CF®680	20253	20263	20273	20384
CF®680R		20842		20841
CF®750			20430	
CF®770	20254	20264	20274	20385

Goat Anti-Human Isotype-Specific Antibodies

2 mg/mL, unit size: 0.25 mL or 50 uL

Conjugate	Goat anti-human IgA (alpha chain)	Goat anti-human IgM (mu chain)
CF®488A	20428	20347
CF®594	20429	20348
CF®640R		20349
CF®633	20427	
CF®647		20346
CF®680		20384

See more highly cross-adsorbed secondaries on the previous page; see p. 20 for single-label antibody conjugates for STORM.

Tyramides & Signal Amplification Kits

Tyramide signal amplification (TSA), sometimes called catalyzed reporter deposition (CARD), is a highly sensitive method enabling the detection of low-abundance targets immunofluorescence applications. For multiplexing, TSA not only facilitates detection of low-abundance targets, but also simplifies antibody panel design since primary antibodies of choice may be used, irrespective of host species or isotype.

In TSA, horseradish peroxidase (HRP) converts a labeled tyramide substrate into a highly reactive form that can covalently bind to tyrosine residues on proteins at or near the HRP-conjugate. This generates high density tyramide labeling and is the reason for the exceptional sensitivity of this system. Because the label is covalently linked to the sample, the antibodies can be stripped off without affecting signal, allowing multiple rounds of staining for multiplex detection using antibodies from the same host species.

We offer CF® dye and other tyramide conjugates with a wide color selection, plus easy-to-use kits and reaction buffer.

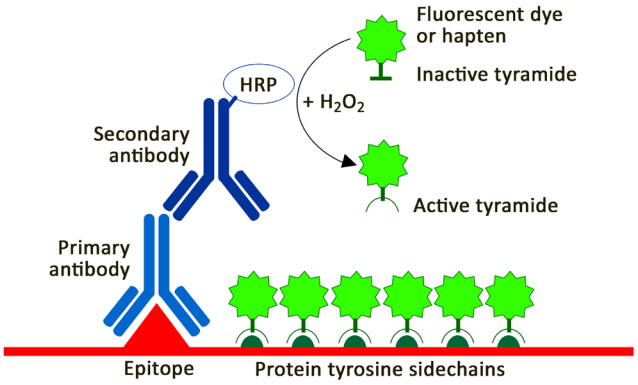


Figure 1. Illustration of tyramide signal amplification. A cell or tissue sample is labeled with primary and secondary antibody using conventional methods. The horseradish peroxidase (HRP), conjugated to the secondary antibody, catalyzes the conversion of labeled tyramide into a reactive radical. The tyramide radical then covalently binds to nearby tyrosine residues, providing high-density labeling.

Advantages of Tyramide Signal Amplification

- Detect low-abundance targets
- ICC, IHC, and FISH-compatible
- Sensitivity up to 100-fold that of conventional methods
- Similar workflow to conventional staining methods
- Use less antibody
- Simplify primary antibody panel design for multiplexing

Tyramide Signal Amplification Kits

Everything you need for the tyramide labeling reaction

- Biotin tyramide or one of six CF® dye tyramides
- HRP conjugate: goat anti-mouse, goat anti-rabbit, or streptavidin
- Tyramide Amplification Buffer Plus
- BSA (for blocking buffer preparation)

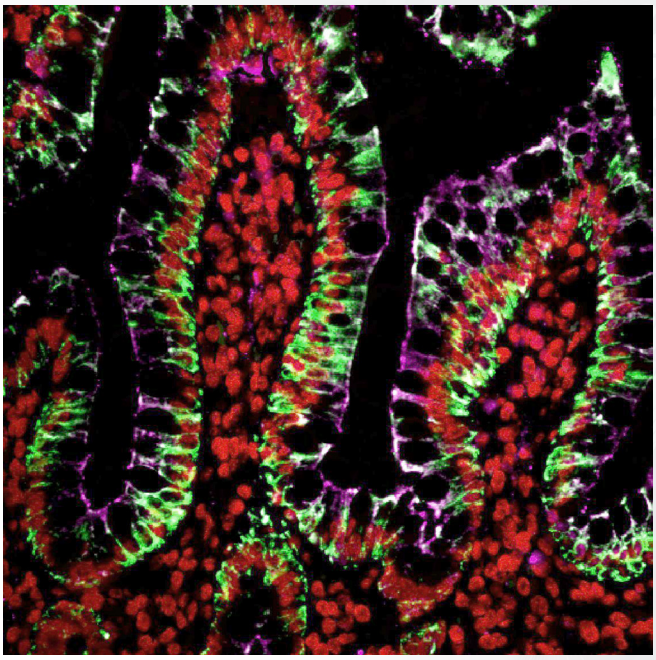


Figure 2. Multiplex tyramide labeling of human colon FFPE tissue. Cytokeratin (pan) was labeled with CF®488A tyramide (cytoskeleton, green); Histone H1 was labeled with Cyanine 555 tyramide (nuclei, red); ZO1 was labeled with CF®640R tyramide (tight junctions, magenta). All primary antibodies were from mouse; secondary antibody was HRP-conjugated goat anti-mouse. Each labeling was performed sequentially, with antibody removal by microwaving between each labeling step.

Tyramide Signal Amplification Kits

Tyramide	Goat anti-mouse HRP	Goat anti-rabbit HRP	Streptavidin HRP
CF®488A	33000	33001	33002
CF®543	33003	33004	33005
CF®568	33006	33007	33008
CF®594	33009	33010	33011
CF®640R	33012	33013	33014
CF®680R	33015	33016	33017
Biotin-XX	33018	33019	33020

Tyramide Amplification Buffer

Cat. No.	Product
22029	Tyramide Amplification Buffer Plus

Tyramides

Dye/Label	Cat. No.
CF®350	92170
CF®405S	92197
CF®405M	96057
CF®405L	92198
CF®430	96053
CF®488A	92171
CF®514	92199
CF®532	96066
CF®543	92172
CF®550R	96077
CF®555	96021
CF®568	92173
CF®583R	96085
CF®594	92174
CF®620R	92194
CF®640R	92175
CF®647	96022
CF®660R	92195
CF®680R	92196
CF®750	96052
Biotin-XX	92176
Fluorescein	96018
DNP	96019
Cyanine 555*	96020

*Structurally identical to Cy®3

Background Suppressors and Accessory Products for IF/IHC/ICC

Lipofuscin autofluorescence in human cerebral cortex sections

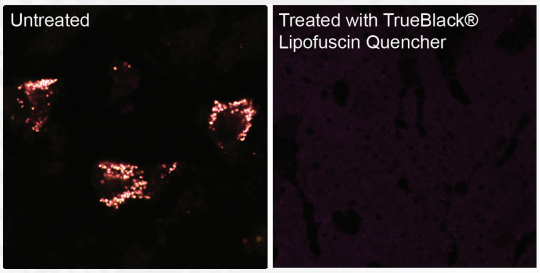


Figure 1. Left: Human brain tissue showing lipofuscin granules with bright, broad-spectrum autofluorescence that appear white in the merged image of the green, red, and far-red channels. Right: Tissue after TrueBlack® treatment, which quenches lipofuscin fluorescence.

Non-specific background from Alexa Fluor® 647 conjugate

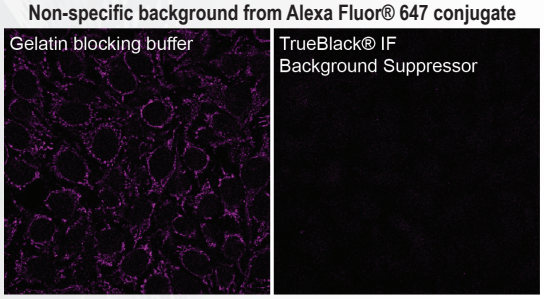


Figure 2. Left: Non-specific signal in HeLa cells caused by binding of negatively charged Alexa Fluor® 647 dye conjugated to secondary antibody. Right: TrueBlack® IF Background Suppressor blocks background from non-specific interactions of charged dyes with biological samples.

Our TrueBlack® line of background quenchers and blocking buffers are designed to reduce background from multiple sources, including tissue autofluorescence, non-specific antibody binding, and non-specific interactions of charged dye conjugates with cells or blotting membranes. We also offer a variety of essential accessory products for immunofluorescence staining.

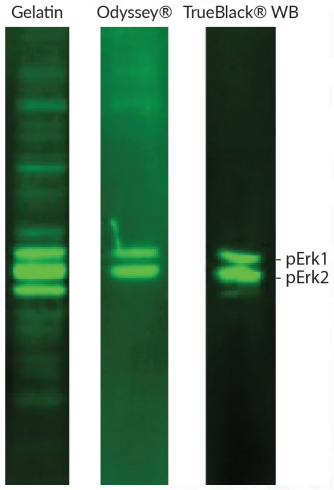


Figure 3. Western detection of phospho-Erk1/2 in PDGF-stimulated NIH-3T3 cell lysate. Membranes were blocked with fish gelatin, LI-COR® Odyssey® TBS Blocking Buffer, or TrueBlack® WB Blocking Buffer. Rabbit anti-pErk1/2 and CF®680R donkey anti-rabbit antibodies were used for detection. TrueBlack® WB Blocking Buffer gave lower background fluorescence and highest specificity.

Cat. No.	Product	Features
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)	• Suppresses background from non-specific antibody binding and charged fluorescent dyes • More efficient than Image-iT® FX, block and permeabilize in just 10 minutes • Non-mammalian blocking agents for broad secondary antibody compatibility • For immunofluorescence on cells or tissue sections
23013	TrueBlack® WB Blocking Buffer Kit	• Blocks as well or better than Odyssey® Blocking Buffer, at a lower price • Reduces non-specific protein bands and background from charged dyes • Compatible with PVDF and nitrocellulose membranes • For visible and near-IR fluorescent westerns
23007	TrueBlack® Lipofuscin Autofluorescence Quencher	• Eliminates lipofuscin autofluorescence with less background than Sudan Black B • Reduces background from other sources • Can be used before or after IF staining
23001	EverBrite™ Mounting Medium	• Excellent protection from photobleaching • Compatible with a wide variety of dyes, including Cy®3, Cy®5, and Alexa Fluor® 647 • Available in wet-set or hard-set formulations • Available with or without DAPI
23002	EverBrite™ Mounting Medium with DAPI	
23003	EverBrite™ Hardset Mounting Medium	
23004	EverBrite™ Hardset Mounting Medium with DAPI	
23008	Drop-n-Stain EverBrite™ Mounting Medium	• EverBrite™ antifade medium in a convenient dropper bottle for pipette-free mounting
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI	
23005	CoverGrip™ Coverslip Sealant	• Replaces nail polish for coverslip sealing • Won't mix with aqueous mounting media
40061	RedDot™2 Far Red Nuclear Counterstain	• Far-red nuclear dye for the Cy®5 channel • More specific than Draq7™
40083	NucSpot® 470 Nuclear Stain,	• Green fluorescent nuclear-specific counterstain for fixed cells or tissues
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses	• Create hydrophobic barriers around tissue sections • Heat-stable to 120°C • Insoluble in aqueous buffers, detergents, alcohol and acetone; can be removed with xylene
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative	• Ready-to-use fixation buffer • Methanol-free, prepared from EM grade paraformaldehyde • No glass ampoules to break, store in the original bottle
22020	10X Phosphate Buffered Saline (PBS) 4L Cubitainer®	• Convenient buffers and blocking agents for immunofluorescence or western
22010	10X Fish Gelatin Blocking Agent	
22014	30% Bovine Serum Albumin Solution	

About Us

At Biotium, we are dedicated to developing cutting-edge fluorescent dyes and life science assays. Innovation is at the very heart of what we do every day. Our efforts have resulted in a growing number of unique and industry-leading fluorescence-based technologies for a wide array of molecular and cellular biology applications. Our products are available in the U.S. through our website, and worldwide through our extensive network of domestic and international distributors.

CF® dyes and Mix-n-Stain™ antibody labeling technology are covered by granted and pending U.S. and international patents. We license our technologies to number of international biotechnology companies, and collaborate with academic laboratories to develop new tools for the ever-changing needs of the research community. **We welcome inquiries about licensing the use of our dyes, technologies, or trademarks; email us at btinfo@biotium.com.**

Biotium implements a Quality System, certified by QAS according to Standard QAS ISO 9001:2015.

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