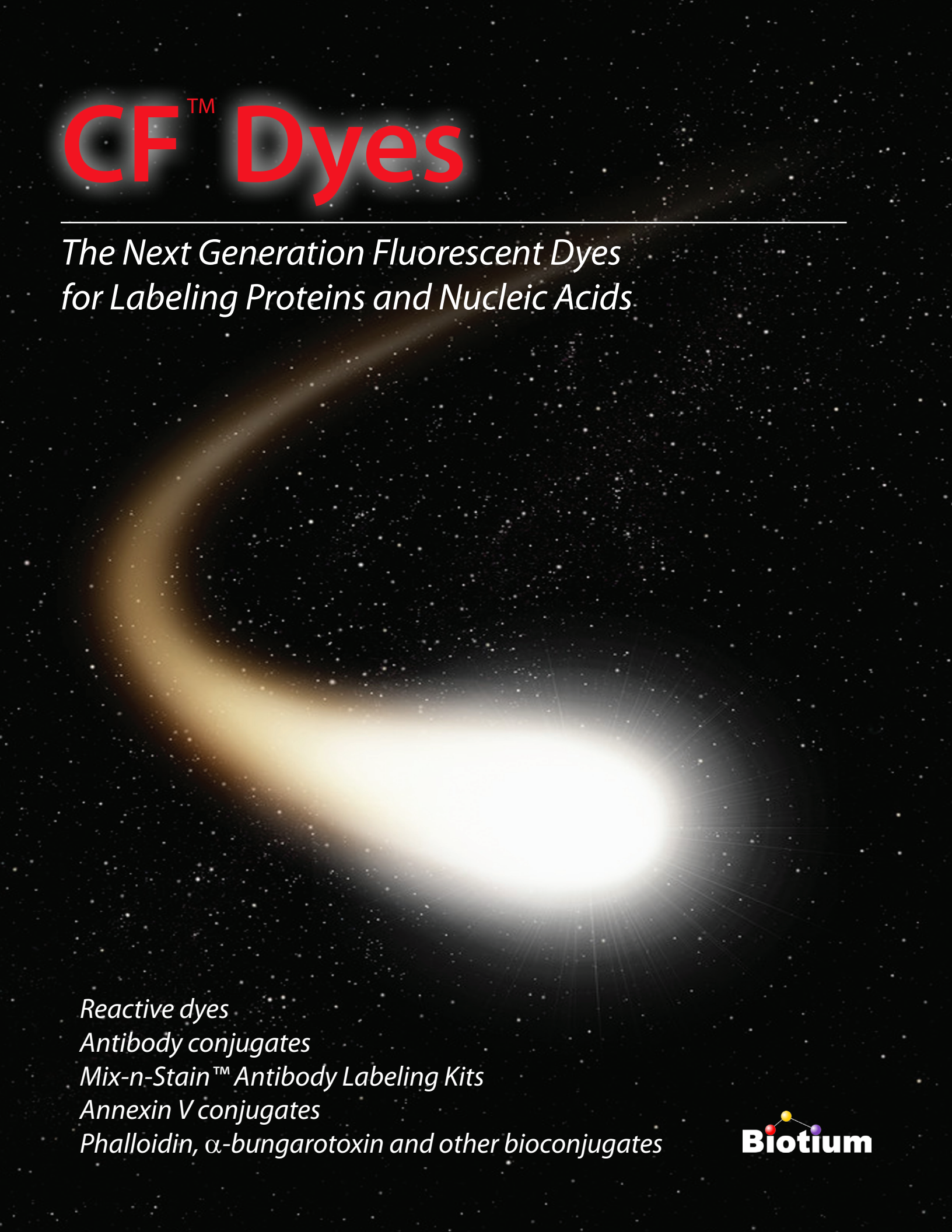
CF™ Dyes

The Next Generation Fluorescent Dyes for Labeling Proteins and Nucleic Acids



Reactive dyes
Antibody conjugates
Mix-n-Stain™ Antibody Labeling Kits
Annexin V conjugates
Phalloidin, α-bungarotoxin and other bioconjugates

Biotium

Table of Contents

CF™ Dyes Quick Reference Table

Visible spectrum

Far-red

Near-infrared

CF™ Dye	λ_{Ex} (nm)	λ_{Em} (nm)	Excitation source*	Replacement for	Advantages	Page
CF™350	347	448	UV	Alexa Fluor® 350, AMCA, DyLight™ 350	• Brightest blue fluorescent conjugates for 350 nm excitation • Highly water-soluble and pH insensitive	6
CF™405S	404	431	405 nm laser	Alexa Fluor® 405, Cascade Blue®, DyLight™ 405	• Better compatibility with common instruments • Highly water-soluble and pH-insensitive	7
CF™405M	408	452	405 nm laser	BD Horizon™ V450, eFluor® 450, Pacific Blue®	• More photostable than Pacific Blue® dye • Less spill-over in the 525/50 green channel • Highly water-soluble	8
CF™488A	490	515	488 nm laser	ATTO™ 488, Alexa Fluor® 488, Cy™2, DyLight™ 488, FAM, FITC, Fluorescein	• Yields biologically more specific antibody conjugates and has less spill-over fluorescence in the red channel than Alexa Fluor® 488 • Extremely photostable • Highly water-soluble and pH-insensitive	9
CF™543	541	560	532, 543, or 546 nm laser	Alexa Fluor® 546, Tetramethylrhodamine (TAMRA)	• Significantly brighter than Alexa Fluor® 546 • Highly water-soluble and pH-insensitive	10
CF™555	555	565	532, 543, 546,, 555, or 568 nm laser	Alexa Fluor® 555, ATTO 550, Cy™3, DyLight™549, TRITC	• Brighter and more photostable than Cy™3	11
CF™568	562	583	532, 543, 546, 555, or 568 nm laser	Alexa Fluor® 568, ATTO 565, Rhodamine Red	• Optimal for the 568 nm line of the Ar-Kr mixed-gas laser • Brighter and more photostable than Alexa Fluor 568	12
CF™594	593	614	532, 543, 546, 555, or 568 nm laser	Alexa Fluor® 594, ATTO 594, DyLight™ 594, Texas Red™	• Yields the brightest conjugates among spectrally similar dyes • Extremely photostable	13
CF™620R	617	639	633 or 635 nm laser	LightCycler® Red 640	• Highly fluorescent • Extremely photostable and highly water-soluble	14
CF™633	630	650	633 or 635 nm laser	Alexa Fluor® 633, Alexa Fluor® 647, Cy™5, DyLight™ 633	• Yields the brightest antibody conjugates among spectrally similar dyes using 633 nm He-Ne laser or the 635 nm red diode laser • Far more photostable than Alexa Fluor® 647 • Highly water-soluble	15
CF™640R	642	662	633, 635, or 640 nm laser	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 • Very water-soluble and pH-insensitive	16
CF™647	650	665	633, 635, or 640 nm laser	Alexa Fluor® 647, ATTO 647N, Cy™5, DyLight™ 649	• Brighter and more photostable than Cy™ 5 • Highly water-soluble and pH-insensitive	17
CF™660C	667	685	633, 635, or 640 nm laser	Alexa Fluor® 660	• Much brighter and more photostable than Alexa Fluor® 660 • Highly water-soluble and pH insensitive	18
CF™660R	663	682	633, 635, or 640 nm laser	Alexa Fluor® 660	• Brighter than Alexa Fluor® 660 • The most photostable 660 nm dye • Highly water-soluble and pH insensitive	18
CF™680	681	698	680 or 685 nm laser	Alexa Fluor® 680, Cy™5.5, DyLight™ 680, IRDye® 680LT	• The brightest among spectrally similar 680 nm dyes • Superior signal-to-noise ratio in immunostaining • Highly water-soluble and pH-insensitive • Compatible with Li-COR Odyssey System	19
CF™680R	680	701	680 or 685 nm laser	Alexa Fluor® 680, Cy™5.5, DyLight™ 680, IRDye® 680LT	• The most photostable 680 nm dye • Suitable for labeling nucleic acids and small biomolecules • Highly water-soluble and pH-insensitive • Compatible with Li-COR Odyssey System	19
CF™750	755	777	680 or 685 nm laser	Alexa Fluor® 750, Cy™7, DyLight™750, APC-Alexa Fluor® 750, IRDye® 750	• Exceptionally bright and stable • Less immunogenic than competing dyes • Better signal-to-noise ratio compared to APC-Alexa Fluor® 750 tandem dye with 633 nm excitation	20-21
CF™770	770	797	785 nm laser	DyLight™ 800, IRDye® 800CW	• Exceptionally bright and stable • Less immunogenic than competing dyes • Compatible with Li-COR Odyssey System	20-21
CF™790	784	806	785 nm laser	Alexa Fluor® 790	• Exceptionally bright and stable • Less immunogenic than competing dyes	20-21

* Most dyes can be excited by a UV light source for epifluorescence microscopy. Wavelengths longer than ~650 nm are not visible to the human eye.

CF™ Dyes
Overview

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 the following novel features.

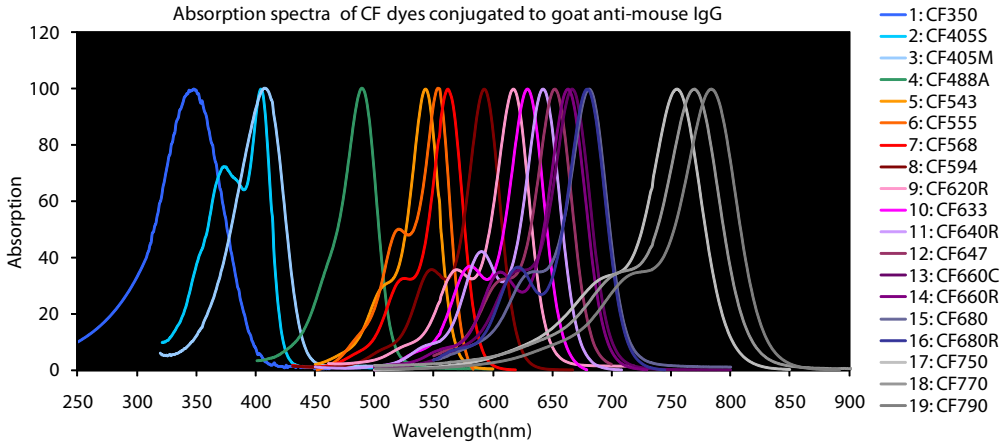
New rhodamine chemistry

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 to the far-red region and even more challenging in the near-IR region, especially for water-soluble dyes 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 a key element in the development of many of our CF™ dyes, particularly our far-red CF™ dyes, which are not only bright and water-soluble but also extremely photostable.

Unrivaled near-IR 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 and poor fluorescence quantum yield. To overcome the problems, many commercial near-IR dyes, such as the near-IR Alexa Fluor® dyes, DyLight® dyes and IRDyes®, 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 (iP), which is essential for maintaining specific antibody-antigen interaction. With this insight, Biotium scientists devised a revolutionary new approach to near-IR dye design that results in superior physical properties of the dyes without introducing an excessive amount of negative charge.

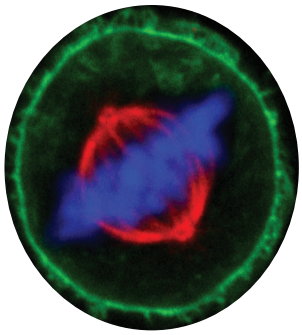
Biotium's near-IR CF™ dyes are based on the core structure of either cyanine dyes or rhodamine dyes. Those core structures are modified such that the intramolecular mobility of the dyes is restricted, which leads to higher quantum yield and better water solubility without adding excessive charge. As a result, near-IR CF™ dyes are much brighter and more photostable than any other near-IR dyes. Most importantly, antibodies labeled with near-IR CF dyes™ give far better signal-to-noise ratio in immunostaining compared with antibody conjugates prepared with other commercial near-IR dyes.



CF™ Dyes *Overview*

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® dyes, DyLight™ dyes and IRDyes® 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 (Alexa Fluor 488 microscale labeling kit product information sheet, Invitrogen). 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 in many of the Alexa Fluor dyes. Accordingly, CF™ dye SE products generally give consistently higher labeling efficiency, thus providing users a better value.

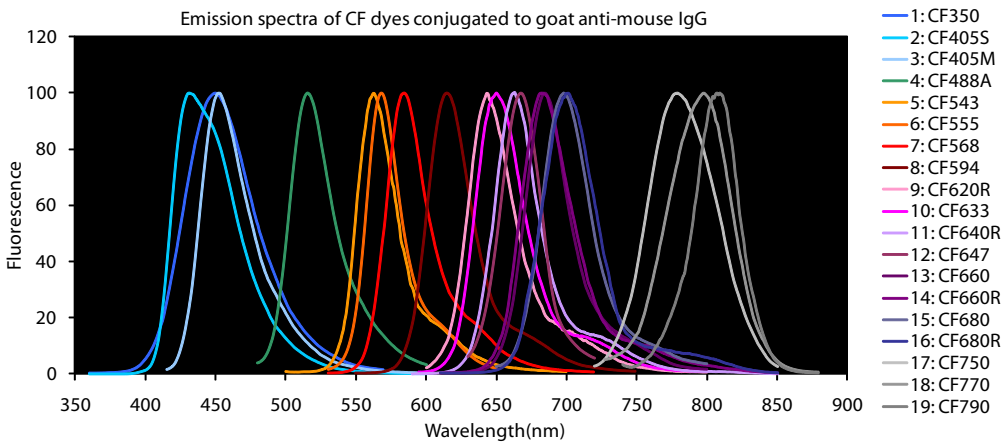


HeLa cell stained with CF633 Mix-n-Stain-labeled anti-tubulin antibody and CF488A phalloidin. See pages 22 and 24 for more information.

Mix-n-Stain™ antibody labeling technology

Biotium has developed a breakthrough antibody labeling technology with CF™ dyes — Mix-n-Stain™ antibody labeling kits. With this technology, you merely need to mix your antibody with the reaction buffer and CF™ dye provided in the kit, and in 30 minutes you will have an optimally labeled CF™ dye-antibody conjugate ready for immunostaining. The labeling technology provides unprecedented convenience for multicolor immunostaining, where pre-labeled primary antibodies may not be available while indirect staining via pre-labeled fluorescent secondary antibodies may also be difficult due to cross-interaction among different antibodies.

Biotium currently offers 18 CF™ dyes with additional colors in development. The CF™ dye product line includes reactive CF™ dyes, labeling kits, CF™-labeled secondary antibodies and streptavidin, and many other CF™-labeled biomolecules.



CF™350
A superior UV-excitable blue fluorescent dye

CF™350 is a blue fluorescent dye spectrally similar to the traditional blue fluorescent dye, AMCA. However, compared to AMCA, CF™350 is more water-soluble, more photostable and at least 50% more fluorescent on proteins. CF™350 is at least as bright as Alexa Fluor® 350 (Figure 2) and thus can be a direct replacement for the latter for all applications.

Technical Summary

Abs/Em Maxima: 347/448 nm
Extinction coefficient: 18,000
Molecular weight: ~ 496
Excitation source: UV
Direct replacement for: Alexa Fluor® 350, AMCA, DyLight™ 350

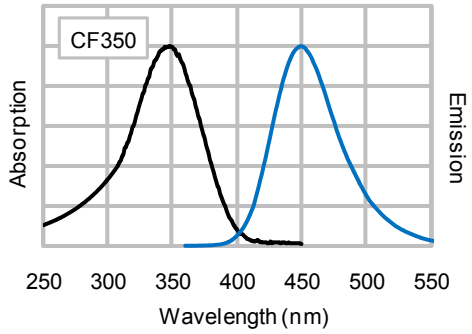


Figure 1. Absorption and emission spectra of CF350 goat anti-mouse conjugate in PBS.

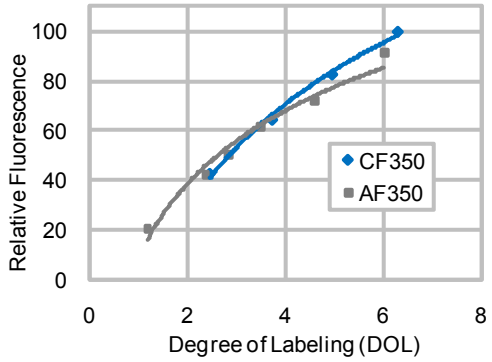


Figure 2. Relative fluorescence of CF350 and Alexa Fluor 350 (AF350) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

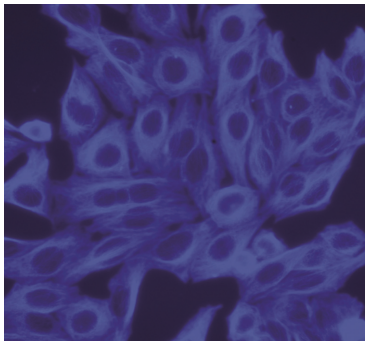


Figure 3. HeLa cells stained with mouse anti-tubulin followed by CF350 goat anti-mouse IgG.

CF™405S
Outshines Alexa Fluor® 405!

Technical Summary

Abs/Em Maxima: 404/431 nm
Extinction coefficient: 33,000
Molecular weight: ~ 1,169
Excitation laser line: 405 nm
Direct replacement for: Alexa Fluor® 405, Cascade Blue®, DyLight™ 405

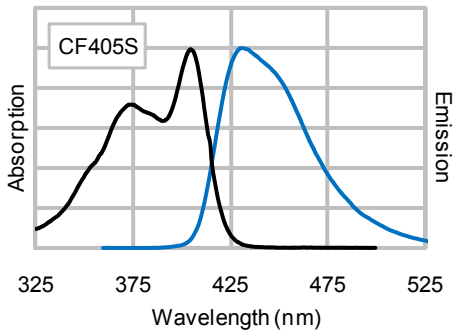


Figure 1. Absorption and emission spectra of CF405S goat anti-mouse conjugate in PBS.

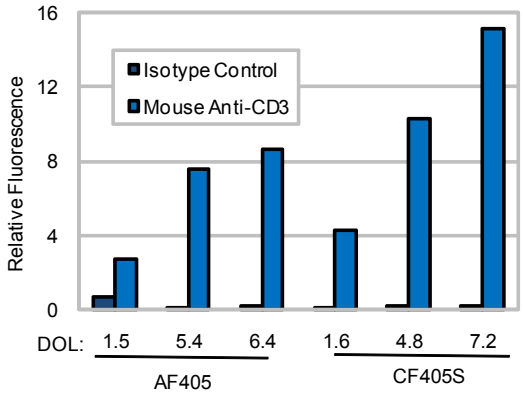


Figure 2. Intracellular staining of Jurkat cells was performed with mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugated to Alexa Fluor 405 (AF405) or CF405S. Fluorescence was analyzed on a BD LSR II flow cytometer with 405 nm excitation and 450/50 nm emission filter. Bars represent the relative fluorescence of the geometric means of the cell populations.

CF™405S is a blue fluorescent dye with an absorption peak wavelength that nearly coincides with the 405 nm blue diode laser line (Figure 1). In addition, the emission peak wavelength of the dye well centers within the blue detection window of BD flow cytometers. As a result, flow cytometry analysis using CF™405S dye results in much brighter signal compared to Alexa Fluor™ 405 (Figure 2).

Advantages

- Much brighter than Alexa Fluor® 405 due to better compatibility with both excitation and emission windows on common instruments
- Highly water-soluble
- pH-insensitive

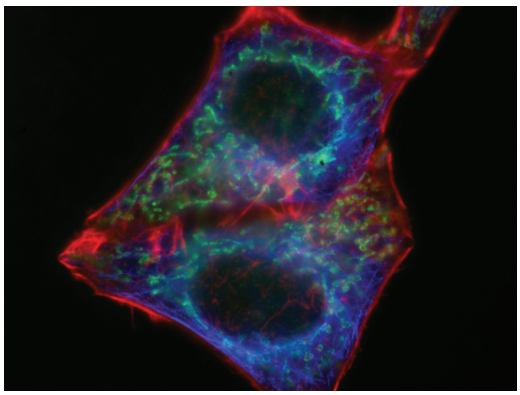


Figure 3. HeLa cells stained with mouse anti-tubulin followed by CF405S goat anti-mouse IgG (blue) and rabbit anti-COX IV (mitochondrial marker) followed by CF488A donkey anti-rabbit IgG (green). Actin filaments are stained with CF555 phalloidin conjugate (red).

CF™405M

A blue fluorescent dye with superior photostability

CF™405M is a blue fluorescent dye spectrally similar to Pacific Blue® dye, but with a narrower emission peak (Figure 1), resulting in less fluorescence spillover in the green channel. CF™405M is as bright as Pacific Blue® dye (Figure 2) but is significantly more photostable (Figure 3), making CF™405M a superior choice in multi-color detection applications.

Technical Summary

Abs/Em Maxima: 408/452 nm

Extinction coefficient: 41,000

Molecular weight: ~ 503

Excitation laser line: 405 nm

Direct replacement for: Pacific Blue®, BD Horizon™ V450

Advantages

- More photostable than Pacific Blue® dye
- Less spillover in the 525/50 green channel
- Highly water-soluble

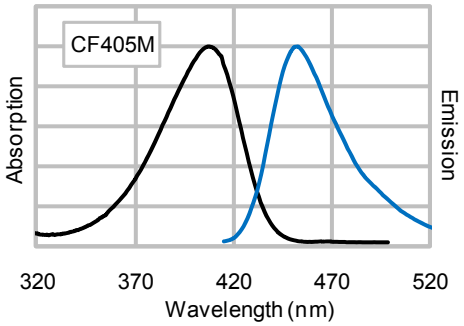


Figure 1. Absorption and emission spectra of CF405M goat anti-mouse conjugate in PBS.

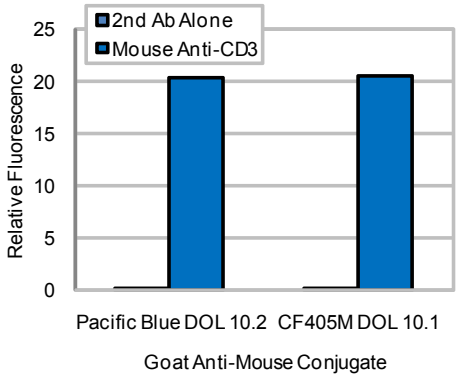


Figure 2. Intracellular staining of Jurkat cells was performed with mouse anti-CD3 or no primary antibody followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD LSR II flow cytometer with 405 nm excitation and 450/50 nm emission filter. Bars represent the relative fluorescence of the geometric means of the cell populations.

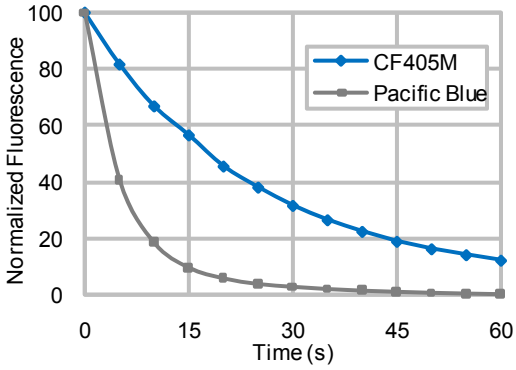


Figure 3. Photostability of CF405M and Pacific Blue. CF405M and Pacific Blue dye solutions were continuously exposed to mercury arc lamp microscope excitation with a DAPI filter set. Images were captured every 5 seconds for one minute. Fluorescence intensity was normalized to time 0.

CF™488A

A superior green fluorescent dye

Technical Summary

Abs/Em Maxima: 490/515 nm

Extinction coefficient: 70,000

Molecular weight: ~710

Excitation laser line: 488 nm

Direct replacement for: Alexa Fluor® 488, DyLight™488, fluorescein (aka FITC, FAM), Cy™2

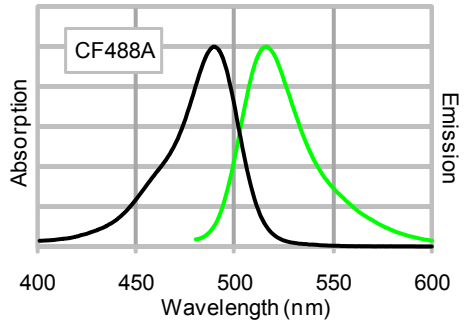


Figure 1. Absorption and emission spectra of CF488A goat anti-mouse conjugate in PBS.

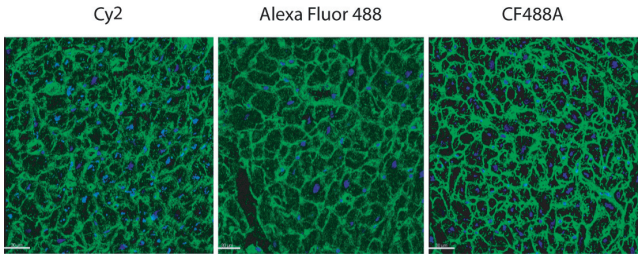


Figure 2. Cryosections of human heart stained with rabbit anti-fibronectin followed by goat anti-rabbit IgG conjugated to Cy2, Alexa Fluor 488, and CF488A, respectively. Courtesy of Dr. Sawa Kostin at Max-Planck-Institute für Herz- und Lungenforschung (W.G. Kerckhoff-Institut) in Bad Nauheim, Hessen, Germany.

CF™488A is a green fluorescent dye optimally excitable by the 488 nm argon laser line. Under common detection conditions, CF™488A is at least as bright as Alexa Fluor® 488, but with a shorter emission wavelength (Figure 1) than Alexa Fluor® 488 and fluorescein, resulting in less fluorescence spill-over in the red channel in multi-color applications. However, a major advantage of CF™488A over Alexa Fluor® 488 is that antibody conjugates prepared from the former are biologically more specific. Alexa Fluor® 488 carries multiple negative charges, which can significantly change the isoelectric point of the proteins the dye labels and consequently alter the specificity of the protein conjugates. CF™488A, on the other hand, is minimally charged. Thus, antibody conjugates prepared from the dye ensure biological detection with high signal-to-noise ratio (Figure 2).

Advantages

- Minimally charged dye reduces non-specific binding of antibody conjugates
- Less spillover fluorescence in the red channel than Alexa Fluor® 488
- Extremely photostable
- Highly water-soluble and pH-insensitive
- Superior stability and labeling efficiency of reactive dyes

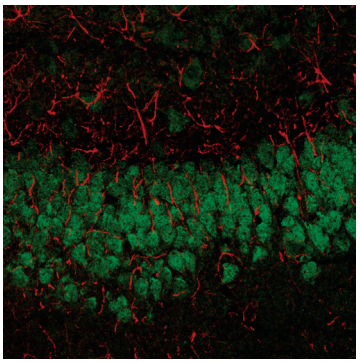


Figure 3. Neuronal nuclei in frozen section of rat hippocampus stained with CF488A-labeled anti-NeuN antibody (green). Glia are stained red with rabbit anti-GFAP and CF555 donkey anti-rabbit antibodies.

CF™543

An orange fluorescent dye ideal for the 543 nm laser

CF™543 is an orange colored fluorescent dye with absorption and emission maxima at 541 and 560 nm, respectively (Figure 1). The dye is based on a new class of rhodamine dyes invented by Biotium scientists. Unlike traditional rhodamine dyes, CF™543 is not only bright and photostable but also highly water-soluble. As a result, CF™543 antibody conjugates retain excellent solubility and yield the brightest fluorescence among antibody conjugates prepared from spectrally similar dyes. For example, CF™543 labeled goat anti-mouse IgG is significantly brighter than the antibody labeled with Alexa Fluor® 546 at similar degree of labeling (Figure 2).

Technical Summary

Abs/Em Maxima: 541/560 nm

Extinction coefficient: 100,000

Molecular weight: ~ 870

Excitation laser line: 532 nm, 543 nm, or 546 nm

Direct replacement for: Alexa Fluor® 546, Tetramethylrhodamine (TAMRA).

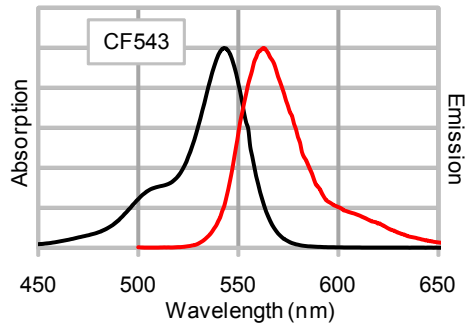


Figure 1. Absorption and emission spectra of CF543 goat anti-mouse conjugate in PBS.

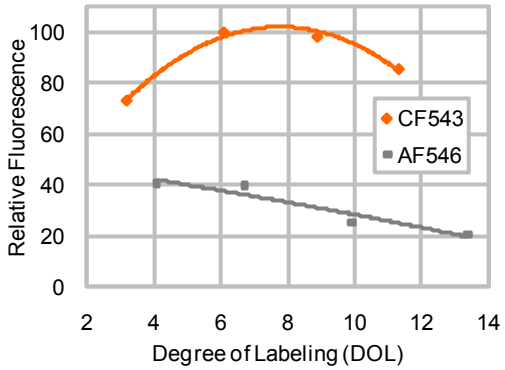


Figure 2. Relative fluorescence of CF543 and Alexa Fluor 546 (AF546) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

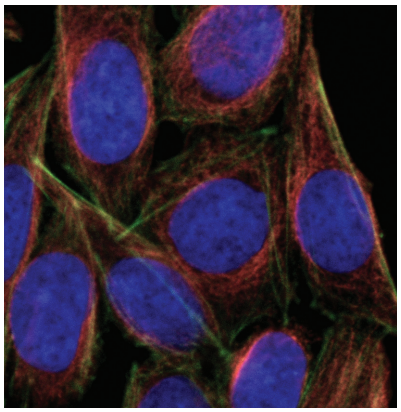


Figure 3. HeLa cells stained with mouse anti-tubulin followed by CF543 goat anti-mouse IgG (red). Actin filaments are stained with CF488A phalloidin conjugate (green) and nuclei are stained with RedDot2 far red nuclear counterstain (blue).

CF™555

A bright and photostable orange-red dye

Technical Summary

Abs/Em Maxima: 555/565 nm

Extinction coefficient: 150,000

Molecular weight: ~ 901

Excitation laser line: 532 nm or 568 nm

Direct replacement for: Alexa Fluor®555, ATTO550, Cy™3, DyLight®549, Rhodamine (TRITC)

CF™555 is an orange-red fluorescent dye spectrally similar to Cy3 and Alexa Fluor®555. Like Alexa Fluor®555, CF™555 is much brighter and more photostable than Cy™3.

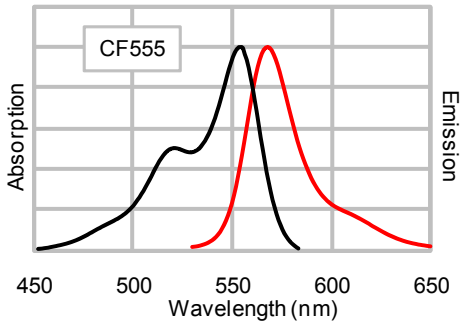


Figure 1. Absorption and emission spectra of CF555 goat anti-mouse conjugate in PBS.

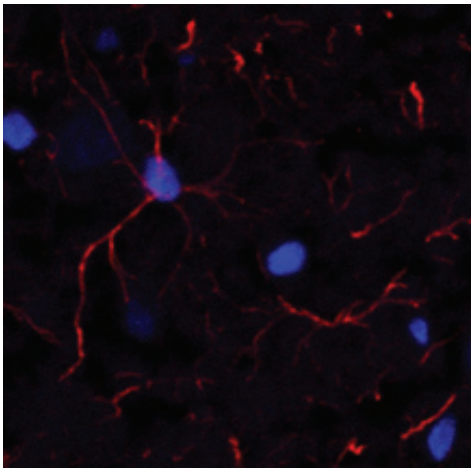


Figure 2. Glial cells in frozen section of rat brain stained with rabbit anti-GFAP and CF555 donkey anti-rabbit antibodies. Nuclei are stained blue with DAPI.

CF™568
Outshines Alexa Fluor®568

CF™568 is a red fluorescent dye with an excitation spectrum optimally matching the 568 nm line of the Ar-Kr mixed-gas laser (Figure 1). Antibody conjugates of CF™568 are much brighter than those of Alexa Fluor® 568 (Figure 2). In addition, the photostability of CF™568 is superior to that of Alexa Fluor® 568 (Figure 3), making CF™568 a better choice for demanding applications such as confocal microscopy and single molecule imaging.

Technical Summary

Abs/Em Maxima: 562/583 nm

Extinction coefficient: 100,000

Molecular weight: ~ 714

Excitation laser line: 532 nm or 568 nm

Direct replacement for: Alexa Fluor® 568, ATTO 565, Rhodamine Red

Advantages

- Yields much brighter antibody conjugates than Alexa Fluor® 568
- Extremely photostable
- Highly water-soluble

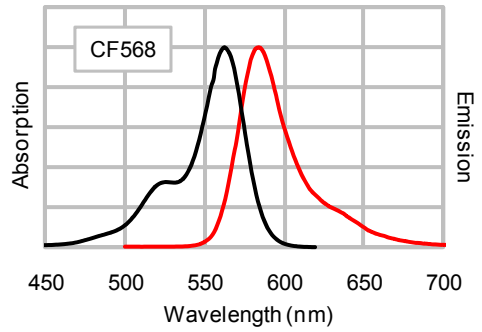


Figure 1. Absorption and emission spectra of CF568 goat anti-mouse conjugate in PBS.

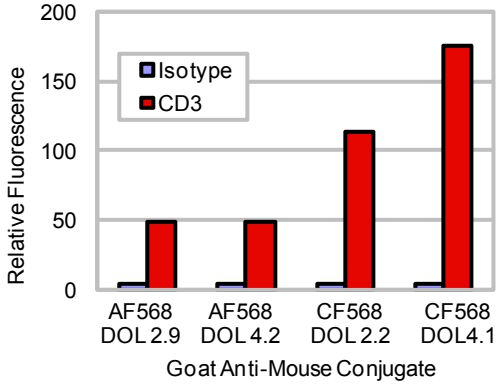


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL2 channel. Bars represent the relative fluorescence of the geometric means of the cell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

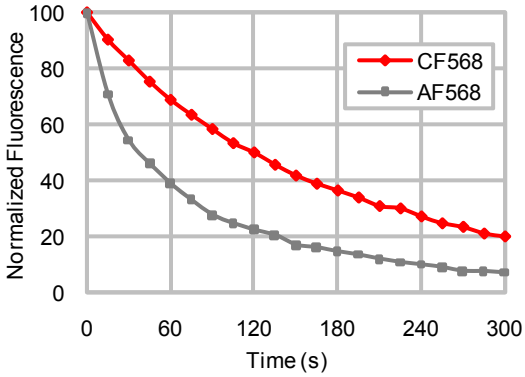


Figure 3. Photostability of CF568 and Alexa Fluor 568 (AF568) streptavidin conjugates. Intracellular staining of Jurkat cells was performed using anti-CD3-biotin followed by streptavidin-CF568 or streptavidin-AF568. Cells were continuously exposed to mercury arc lamp microscope excitation with a Cy3 filter set. Images were captured every 15 seconds for 5 minutes and fluorescence intensity was normalized to time 0.

CF™594

Truly the brightest deep red dye

Technical Summary

Abs/Em Maxima: 593/614 nm

Extinction coefficient: 115,000

Molecular weight: ~730

Excitation laser line: 532 nm, 568 nm or 594 nm

Direct replacement for: Alexa Fluor® 594, ATTO™ 594, DyLight™ 594, Texas Red®

CF™594 is a deep red fluorescent dye spectrally similar to Alexa Fluor® 594 and Texas Red® dye (Figure 1). When conjugated to proteins, CF™594 is significantly brighter than Alexa Fluor® 594 (Figure 2) due to its high quantum yield and exceptional water solubility. CF™594 also has excellent photostability (Figure 3), making it ideal for demanding applications such as confocal microscopy and single molecule imaging. The dye is particularly useful in combination with our blue fluorescent CF™350, green fluorescent CF™488A and far red CF™640R for multi-color imaging.

Advantages

- Yields the brightest antibody conjugates among spectrally similar dyes.
- Extremely photostable
- Highly water-soluble

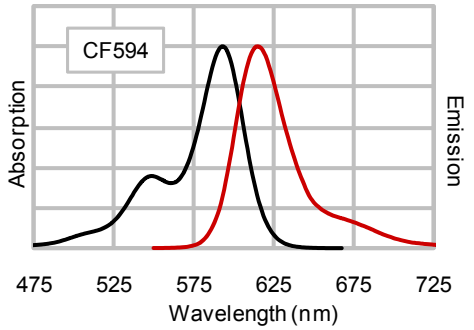


Figure 1. Absorption and emission spectra of CF594 goat anti-mouse conjugate in PBS.

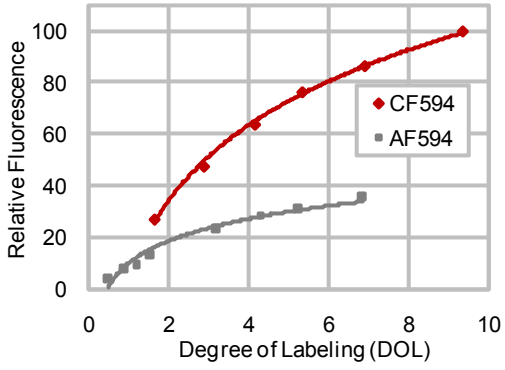


Figure 2. Relative fluorescence of CF594 and Alexa Fluor 594 (AF594) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

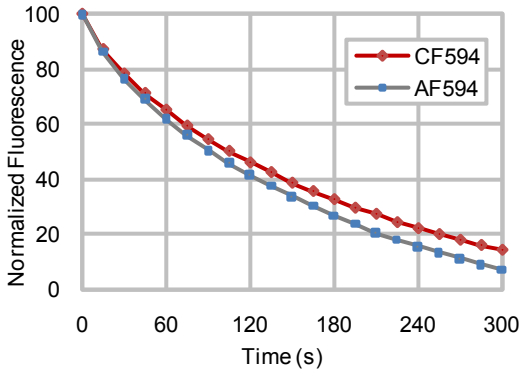


Figure 3. Photostability of CF594 and Alexa Fluor 594 (AF594) goat anti-mouse conjugates. Intracellular staining of Jurkat cells was performed with mouse anti-CD3 followed by CF594 or AF594 goat anti-mouse conjugates. Cells were continuously exposed to mercury arc lamp microscope excitation with a Texas Red filter set. Images were captured every 15 seconds for 5 min and fluorescence intensity was normalized to time 0.

CF™620R
A bright and photostable far red dye

CF™620R dye is a far-red rhodamine-based fluorescent dye. The dye is highly fluorescent and extremely photostable. With its absorption and emission maxima centered at 617 and 639 nm (Figure 1), the dye may be used as an excellent energy acceptor in fluorescence resonance energy transfer (FRET), or used in multi-color detection applications where the excitation and emission windows can be matched with the spectral profiles of the dye for maximal fluorescence collection. The exceptional water solubility of the dye facilitates bioconjugation in aqueous media and better preserves the biological specificity of the conjugates.

Advantages

- Highly water-soluble
- Highly fluorescent
- Extremely photostable

Technical Summary

Abs/Em Maxima: 617/639 nm

Extinction coefficient: 115,000

Molecular weight: ~ 738

Excitation laser line: 633 nm or 635 nm

Direct replacement for: LightCycler® Red 640

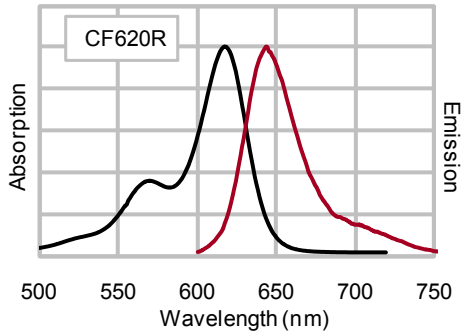


Figure 1. Absorption and emission spectra of CF620R goat anti-mouse conjugate in PBS.

CF™633
The best dye for 633/635 laser lines

Technical Summary

Abs/Em Maxima: 630/650 nm

Extinction coefficient: 100,000

Molecular weight: ~820

Excitation laser line: 633 nm or 635 nm

Direct replacement for: Alexa Fluor® 633, Alexa Fluor® 647, Cy™5, DyLight™ 633, DyLight™ 649

With its 630 nm absorption peak (Figure 1), CF™633 can be optimally excited by the 633 nm He-Ne laser or the 635 nm red diode laser. Despite its shorter emission wavelength, CF™633 is still significantly brighter than Alexa Fluor® 647 (Figure 2). The most important advantage of CF™633 is its unmatched photostability (Figure 3).

Advantages

- Yields the brightest antibody conjugates among spectrally similar dyes when excited by the 633 nm He-Ne laser or the 635 nm red diode laser
- Far more photostable than Alexa Fluor® 647
- Highly water-soluble

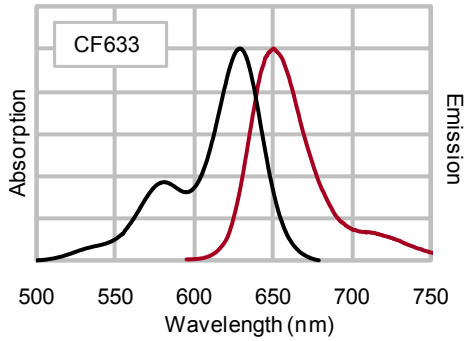


Figure 1. Absorption and emission spectra of CF633 goat anti-mouse conjugate in PBS.

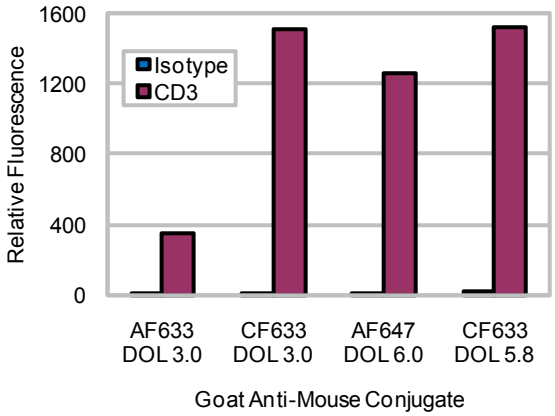


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel. Bars represent the relative fluorescence of the geometric means of the cell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

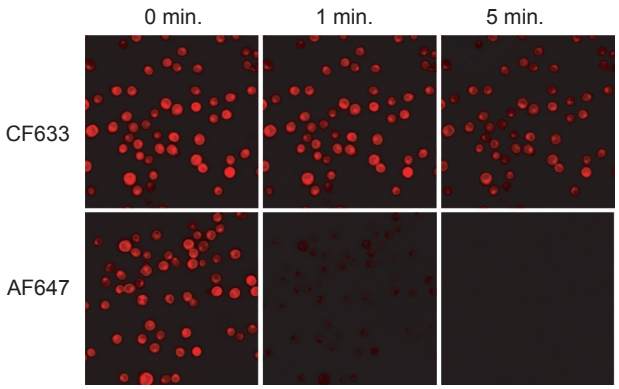


Figure 3. Relative photostability of CF633 and Alexa Fluor 647 goat anti-mouse conjugates. Jurkat cells were fixed, permeabilized and stained with rabbit anti-CD3 (Abcam) followed by CF633 or Alexa Fluor 647 goat anti-rabbit IgG conjugates. Cells were imaged using a mercury arc lamp microscope equipped with a Cy5 filter set and CCD camera. Sequential images were captured at 0, 1, and 5 minutes.

CF™640R
A highly photostable far-red dye

CF™640R is a rhodamine-based far-red fluorescent dye with excitation and emission maxima very similar to those of Cy™5 and Alexa Fluor® 647 (Figure 1). CF™640R is much brighter than Cy™5 and at least as bright as Alexa Fluor® 647 (Figure 2). A major advantage of CF™640R over Cy™ 5 and Alexa Fluor® 647 is its exceptional photostability (Figure 3). Cy™5 and Alexa Fluor® 647 are cyanine-based dyes and, like other cyanine dyes in general, have intrinsically poor photostability. CF™640R is also far brighter and more photostable than Atto™ 647N, another spectrally similar dye frequently used in single-molecule imaging. The combination of excellent brightness and photostability makes CF™640R ideal for confocal microscopy, single-molecule imaging and other demanding applications.

Advantages

- Has the best photostability among dyes with Cy™5-like spectra
- Yields highly fluorescent protein conjugates
- Highly water-soluble and pH-insensitive

Technical Summary

Abs/Em Maxima: 642/662 nm

Extinction coefficient: 105,000

Molecular weight: ~ 832

Excitation laser line: 633 nm, 635 nm or 640 nm

Direct replacement for: Alexa Fluor® 647, ATTO™ 647N, Cy™5, DyLight™649

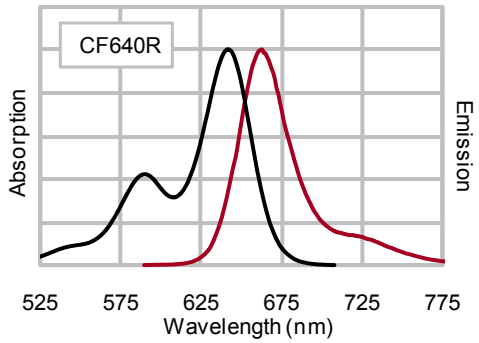


Figure 1. Absorption and emission spectra of CF640R goat anti-mouse conjugate in PBS.

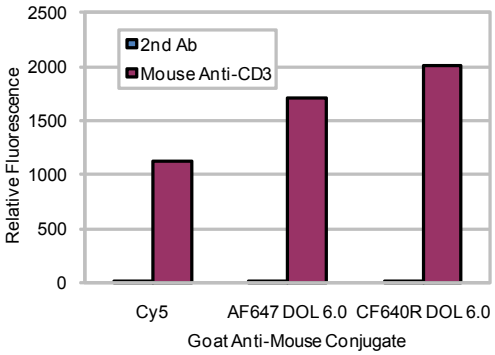


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel. Bars represent the relative fluorescence of the geometric means of the cell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

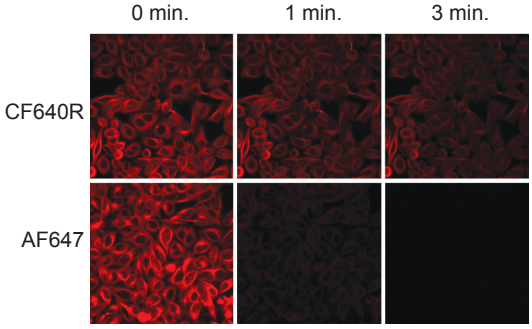


Figure 3. Photostability comparison between CF640R and Alexa Fluor 647 (AF647). HeLa cells were stained with anti-tubulin antibody conjugates of CF640R or Alexa Fluor 647 (AF647). Cells were continuously illuminated by a mercury arc lamp microscope and sequential images were captured at 0, 1, and 3 minutes.

CF™647
A superior far red dye

Technical Summary

Abs/Em Maxima: 650/665 nm

Extinction coefficient: 240,000

Molecular weight: ~ 836

Excitation laser line: 633 nm, 635 nm or 640 nm

Direct replacement for: Cy™5, Alexa Fluor® 647, DyLight™ 649

CF™647 is a cyanine-based far-red fluorescent dye spectrally similar to Cy™5 and Alexa Fluor® 647 (Figure 1). Like Alexa Fluor 647, CF™647 is much brighter than Cy™5.

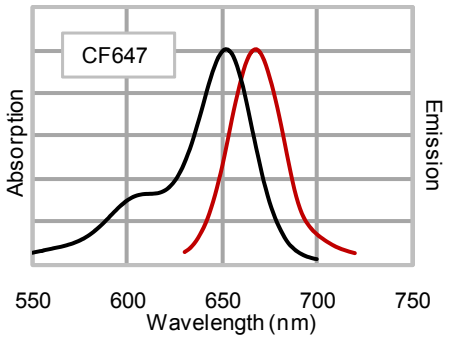


Figure 1. Absorption and emission spectra of CF647 goat anti-mouse conjugate in PBS.

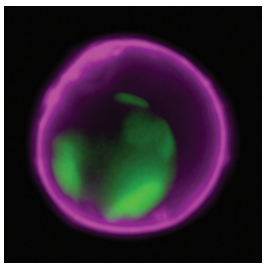


Figure 2. Jurkat cells were treated with staurosporine to induce apoptosis and stained with NucView™488 Caspase-3 Substrate (green) and CF647 Annexin-V (magenta).

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 nm, 635 nm or 640 nm

Direct replacement for: Alexa Fluor® 660, Allophycocyanin (APC)

CF™660R

Abs/Em Maxima: 663/682 nm

Extinction coefficient: 100,000

Molecular weight: ~ 888

Excitation laser line: 633 nm, 635 nm or 640 nm

Direct replacement for: Alexa Fluor® 660, Allophycocyanin (APC)

CF™660C and CF™660R are two spectrally similar fluorescent dyes that emit fluorescence at about 685 nm in the borderline spectral region between far-red and near-IR (Figure 1). The two dyes are spectrally similar to Alexa Fluor® 660 but with far superior performance. CF™660C protein conjugates are several fold brighter than Alexa Fluor® 660 (Figure 2). In addition, CF™660R is exceptionally photostable (Figure 3), making it ideal for confocal microscopy and other demanding applications.

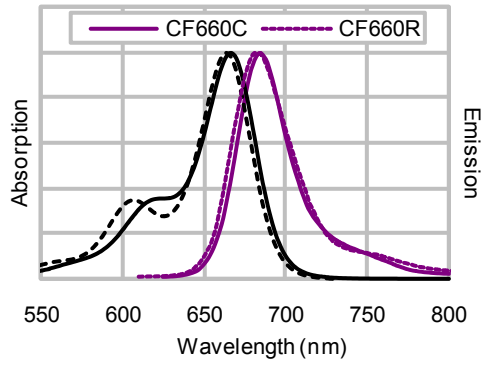


Figure 1. Absorption and emission spectra of CF660C and CF660R goat anti-mouse conjugates in PBS.

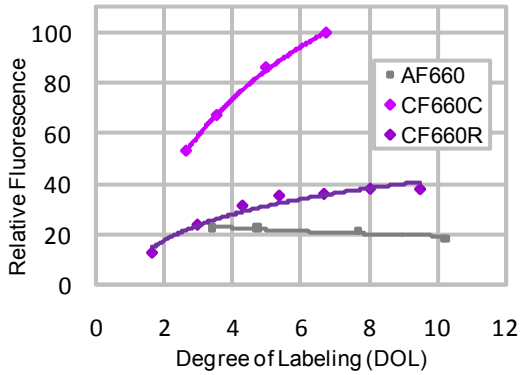


Figure 2. Relative fluorescence of CF660, CF660R and Alexa Fluor 660 (AF660) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

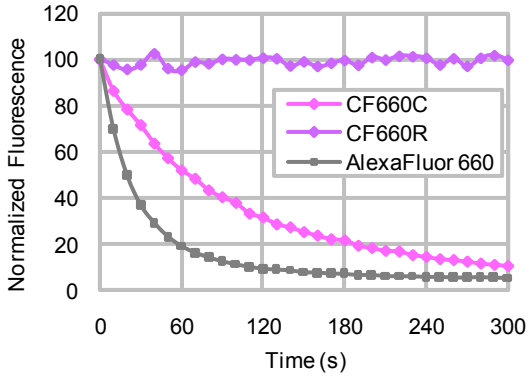


Figure 3. Photostability of CF660C, CF660R, and Alexa Fluor 660 (AF660) goat anti-mouse conjugates. HeLa cells were stained with mouse anti-tubulin followed by CF660C, CF660R or AF660 goat anti-mouse IgG conjugates. Cells were continuously exposed to mercury arc lamp microscope excitation using a Cy5 filter set. Images were captured every 10 seconds for five minutes and fluorescence intensity was normalized to time 0.

CF™680 and CF™680R Dyes
Two outstanding 680 nm-excitable dyes

CF™680 is a highly water-soluble cyanine-based dye with a molecular weight of ~3000. This dye is excellent for labeling antibodies, producing the brightest fluorescence and highest signal-to-noise ratio in immunostaining among spectrally similar dyes. Because of its relatively large molecular size, CF™680 is not recommended for labeling nucleic acids or relatively small biomolecules, for which CF™680R is better suited.

CF™680R is a novel rhodamine-based dye that is highly fluorescent and extremely photostable, making it are ideal for confocal microscopy, single molecule-based imaging and other demanding applications.

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

Direct replacement for: Alexa Fluor® 680, Cy™5.5, IR®Dye 680

CF™680R

Abs/Em Maxima: 680/701 nm

Extinction coefficient: 140,000

Molecular weight: ~ 912

Excitation laser line: 680 nm or 685 nm

Direct replacement for: Alexa Fluor® 680, Cy™5.5, IR®Dye 680

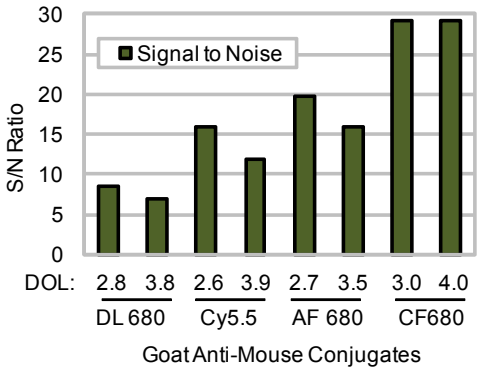


Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-human CD3 antibody or isotype control followed by goat anti-mouse secondary antibody conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel Bars represent the signal-to-noise ratio of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

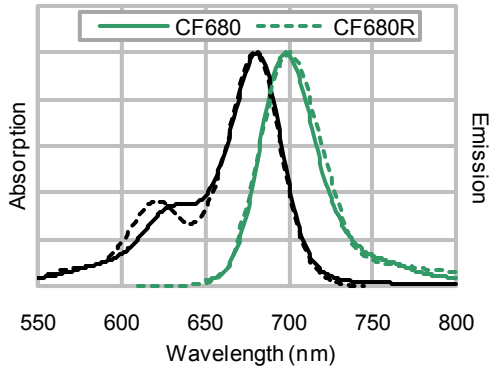


Figure 1. Absorption and emission spectra of CF680 and CF680R goat anti-mouse conjugates in PBS.

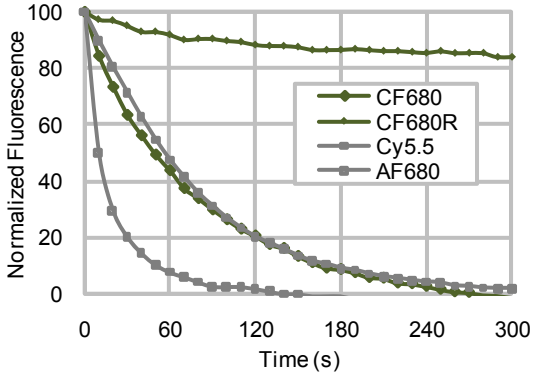


Figure 3. Photostability of far red dye conjugates. Jurkat cells were stained with mouse anti-CD3 followed by the indicated goat anti-mouse IgG conjugates. Cells were continuously exposed to mercury arc lamp excitation with a Cy5 filter set. Images were captured every 10 seconds for 5 minutes and fluorescence intensity was normalized to time 0.

CF™750, CF™770 and CF™790
Unrivaled Near-Infrared Dyes

Technical Summary

CF™750

Abs/Em Maxima: 755/777 nm
Extinction coefficient: 250,000
Molecular weight: ~ 3009
Excitation laser line: 633 nm, 635 nm, 680 nm or 685 nm
Direct replacement for: Alexa Fluor® 750, Cy™7, DyLight™ 750, APC- Alexa Fluor® 750

CF™770

Abs/Em Maxima: 770/797 nm
Extinction coefficient: 220,000
Molecular weight: ~ 3138
Excitation laser line: 785 nm
Direct replacement for: DyLight™ 800, IRDye 800CW

CF™790

Abs/Em Maxima: 784/806 nm
Extinction coefficient: 210,000
Molecular weight: ~ 3267
Excitation laser line: 785 nm
Direct replacement for: Alexa Fluor® 790

Near-IR dyes offer important advantages over traditional visible light dyes. Because cells and tissues produce minimal autofluorescence in the near-IR region, near-IR dyes have the potential to offer highly specific and sensitive detection in complex biological systems. Also, because light with wavelength in the near-IR region has strong tissue penetration, near-IR dyes are ideal for in vivo fluorescence imaging (Figure 2), an emerging field that has advanced rapidly in recent years. Furthermore, near-IR dyes are also excellent for in- or on-cell and membrane-based Western assays (Figure 4).

CF™750, CF™770 and CF™790 are next-generation long wavelength dyes representing a true breakthrough in the field. Other near-IR dyes suffer from problems of limited fluorescence brightness due to excessive dye aggregation and poor stability. As a result of novel molecular engineering by scientists at Biotium, near-IR CF dyes overcome these problems. Even when excited at 633 nm, CF750 is bright enough that it yields better signal to noise ratios in flow cytometry than APC-Alexa Fluor 750, without the poor stability associated with tandem dyes (Figure 3). In addition, near-IR CF dyes are highly water soluble without carrying excessive negative charge, which can increase non-specific binding of antibody conjugates (Figures 3 and 4).

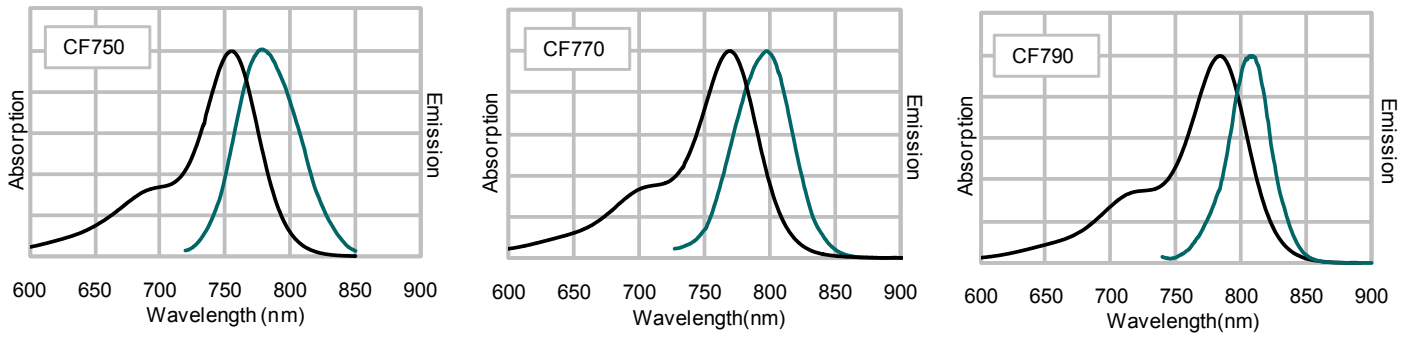


Figure 1. Absorption and emission spectra of near-IR CF dye goat anti-mouse conjugates in PBS.

CF™750, CF™770 and CF™790
Unrivaled Near-Infrared Dyes

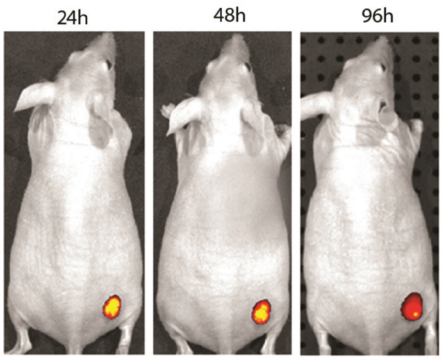


Figure 2. Tumors in mice were imaged using an IVIS imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin conjugated to CF750. Images courtesy of Caliper Life Sciences.

Advantages

- Exceptionally bright and stable
- Ideal for in vivo imaging
- Superior signal-to-noise for bioconjugates

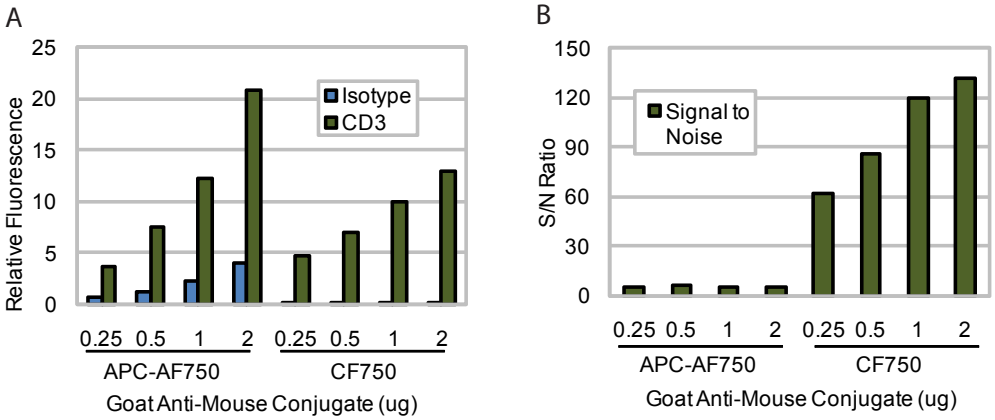


Figure 3. Jurkat cells were stained with isotype or mouse anti-human CD3 antibody followed by goat anti-mouse APC-Alexa Fluor 750 (Invitrogen) or CF750 using the amount of antibody shown. Fluorescence was analyzed using a BD LSR II flow cytometer with 633 nm excitation and 780/60 nm emission filter. A. Bars represent the relative fluorescence values of the geometric means. B. Bars represent signal to noise ratio (CD3 geometric mean/Isotype geometric mean).

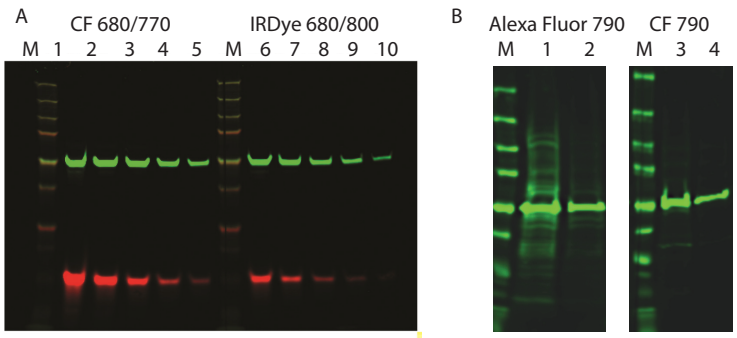


Figure 4. Western blotting with near-IR CF dyes, detected using the Odyssey® infrared imaging system (Li-COR Biosciences). A. Two-fold serial dilutions of HeLa cell lysate (increasing dilution, left-to-right) were probed with rabbit anti-COX IV and mouse anti-tubulin primary antibodies followed by goat anti-rabbit CF680 or IRDye 680 (red) and goat anti-mouse CF770 or IRDye 800 (green). Bands detected with CF dye secondaries show about 3.5-fold higher fluorescence intensity compared to IRDye secondary antibodies. M: Odyssey two-color molecular weight markers (Li-COR). B. Two dilutions of HeLa cell lysate(1, 3: 1X lysate; 2, 4: 1:5 dilution of lysate) were probed with mouse anti-tubulin antibody followed by goat anti-mouse conjugated to Alexa Fluor 790 (1-2) or CF790 (3-4). M: Dylight 680/800 Protein Ladder (Pierce).

Mix-n-Stain™ Antibody Labeling Kits

Label your antibody with CF™ Dyes in only 30 minutes

Advantages

- The simplest antibody labeling protocol available
- Covalently label your antibody in 30 minutes
- No clean up of free dye required
- Tolerates common antibody buffer components

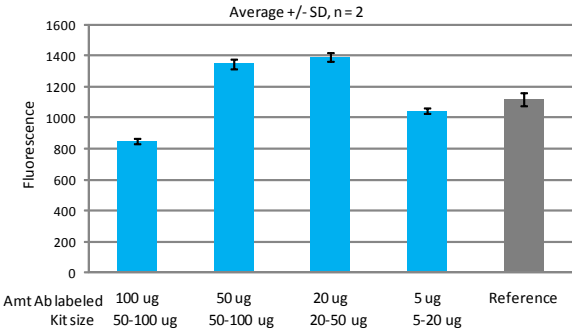


Figure 1. Flow cytometry analysis of Jurkat cells stained with CF™633 Mix-n-Stain labeled mouse anti-human CD3 antibodies (BD cat# 555330). For reference, cells were stained with commercial Alexa Fluor® 647 mouse anti-human CD3 (BD cat# 557706). Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel.

Mix-n-Stain™ CF™ dye antibody labeling kits dramatically simplify the process of preparing fluorescently-labeled antibodies. Simply mix your antibody with the reaction buffer and CF™ dye provided in the kit, a step that takes less than 30 seconds of hands-on time. After 30 minutes incubation, and without a separation step, you will have an antibody conjugate of the CF™ dye of your choice that is comparable to commercially-available fluorescent antibody conjugates (Figure 1). CF™ dyes are covalently linked to the antibody using the Mix-n-Stain™ labeling kits; thus, there is no dye transfer between antibodies or diffusion during multi-color staining. In addition, Mix-n-Stain™ conjugates are stable for at least 6 months at 4ºC in the Storage Buffer provided. Mix-n-Stain™ biotin antibody labeling kits also are available.

Mix-n-Stain™ labeling kits are available for our full spectrum of CF™ dyes in three sizes, for labeling 5-20 ug, 20-50 ug, or 50-100 ug of antibody.

There is no need to calculate how much dye you should use; just mix your antibody with the entire amount of dye provided for optimal labeling. Moreover, the labeling reaction can tolerate the presence of common antibody buffer components such as azide, Tris, BSA and gelatin.

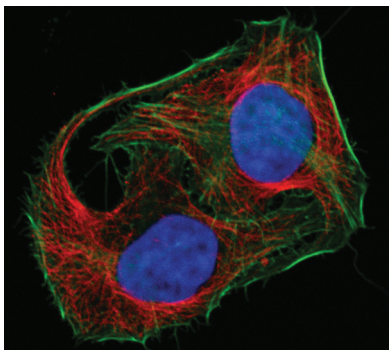


Figure 2. HeLa cells were stained with CF633 anti-tubulin antibody (red) conjugated using the CF633 Mix-n-Stain kit. Actin filaments are stained with CF488A phalloidin (green) and nuclei are stained with DAPI (blue). Images were captured on a Zeiss 510 Meta Confocal microscope.

			Mix-n-Stain™ Kits (CF™ Dye/Catalog number)																	
Reaction size	Unit size	Biotin	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770
5-20 ug antibody	1 labeling	92286	92270	92271	92272	92273	92287	92274	92275	92276	92277	92278	92279	92280	92281	92282	92283	92284	92285
20-50 ug antibody	1 labeling	92266	92250	92251	92252	92253	92267	92254	92255	92256	92257	92258	92259	92260	92261	92262	92263	92264	92265
50-100 ug antibody	1 labeling	92244	92230	92231	92232	92233	92247	92234	92235	92236	92237	92245	92238	92239	92243	92240	92246	92241	92242

CF™ Reactive Dyes and Protein Labeling Kits

CF™ dyes are available in a number of reactive forms for labeling proteins, nucleic acids, and other biomolecules.

- **Succinimidyl ester** (SE) groups label free amines of target molecules, such as lysine residues in proteins. Amine-reactive dyes also have been used in cell viability assays to stably label cells with compromised membrane integrity. CF™ dye SE groups are more resistant to hydrolysis compared to most of the amine-reactive Alexa Fluor® dyes and other reactive dyes, resulting in better stability and superior labeling efficiency. Biotium offers stand-alone CF™ dye succinimidyl esters as well as SE protein labeling kits that include everything you need to perform three labeling reactions of 1 mg protein each.
- **Maleimide** reactive groups can be used to label thiol groups of target molecules, such as cysteine residues in proteins. Maleimide can be used as an alternative to succinimidyl ester (SE) labeling for labeling proteins in cases where SE labeling interferes with a protein's biological activity.
- **Aminooxy** groups readily react with aldehyde or ketone groups to form a stable linkage without the use of a reducing agent.
- **Amine groups** can react with activated carboxylic acid groups of biomolecules.
- **Hydrazide** reactive groups can be used to label target molecules with aldehyde or ketone groups. CF™ dye hydrazides also can be used as fixable fluorescent polar tracers for visualizing neuronal cell morphology and studies of gap junctions.

		Reactive Dyes (CF™ Dye/Catalog number)																		
	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF620R	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770	CF790
Amine	1 mg	92035	92036		92037		92038	92039	92040		92041	92043	92042							
Aminooxy	1 mg	92050	92055	92056	92051			92057	92052		92053	92058			92059		92054			
Hydrazide	1 mg	92151			92152		92153	92154	92158		92156	92157	92136							
Maleimide	1 umol	92020	92030	92021	92022	92044	92023	92024	92025	92033	92026	92034	92027	92028	92031	92029	92032			
Succinimidyl Ester	1 umol	92109	92110	92111	92120	92105	92130	92131	92132	92106	92133	92108	92135	92137	92134	92139	92107	92142	92150	
	0.25 umol																			92155
SE Protein Labeling Kit	3 labelings	92210	92211	92212	92213	92209	92214	92215	92216		92217	92225	92218	92219	92223	92220	92226	92221	92222	

VivoBrite™ Rapid Antibody Labeling Kits for Small Animal Imaging

These labeling kit include our superior near-IR amine-reactive dyes and other components necessary for carrying out antibody labeling, purification and sterile filtration. Available with CF™680 SE, CF™750 SE, or CF™770 SE for three labeling reactions of 1 mg antibody each per kit.

VivoBrite Labeling Kits (CF™ Dye/Catalog number)		
CF680	CF750	CF770
92160	92161	92162

CF™ Dye Bioconjugates

Biotium offers wide selection of CF™ dye-conjugated bioconjugates (Table 1) and related kits (Table 2, next page) for staining of cells, tissue sections and bacteria.

Table 1. CF™ dye bioconjugates

Conjugate	Application
Annexin V	Apoptosis (phosphatidylserine) detection
α-Bungarotoxin	Acetylcholine receptor probe
Bovine serum albumin	In vivo blood flow tracer
Concanavalin A	Carbohydrate probe (lectin)
dUTP	DNA probes, TUNEL assay
Phalloidin	Filamentous actin probe
Streptavidin	Biotin detection
Wheat germ agglutinin	Carbohydrate probe (lectin), bacterial gram stain

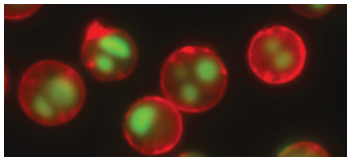


Figure 1. Apoptotic Jurkat cell stained with CF™647 Annexin V (magenta) and NucView™ 488 caspase-3 substrate (green).

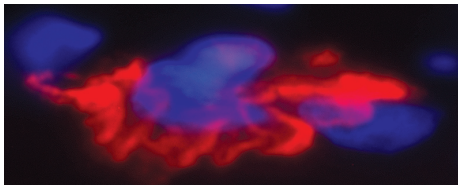


Figure 2. Frozen section of rat skeletal muscle stained with CF633 a-bungarotoxin (red) to detect nicotinic acetylcholine receptors at the neuromuscular junction. Nuclei are stained with DAPI (blue).

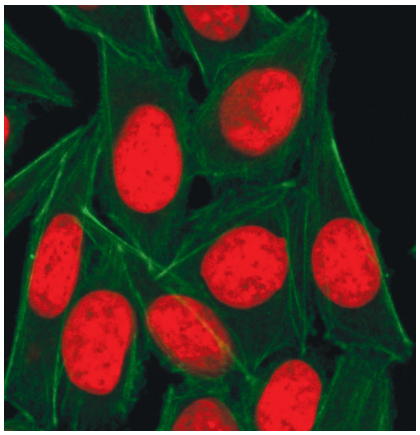


Figure 3. Actin filaments in HeLa cells stained with CF488A phalloidin (green). Nuclei are stained with RedDot2 (red).

		CF™ Dye Bioconjugates (CF™ Dye/Catalog number)														
Conjugate	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660R	CF680	CF680R	CF750
Annexin V, 50 ug/mL in PBS	0.5 mL	29012		29009	29005		29004	29010	29011	29008	29014	29003				
Annexin V, lyophilized solid	25 ug													29007		29006
α-Bungarotoxin	0.5 mg		00002		00005		00018	00006	00007	00009	00004			00008	00003	
Bovine serum albumin	5 x 1 mg				20289				20290		20291			20292		
Concanavalin A	5 x 1 mg	29015			29016				29017	29018	29019					
dUTP	25 nmol		40004		40008	40002		40005	40006		40007					
Phalloidin	300 U	00049		00034	00042	00043	00040	00044	00045	00046	00050	00041	00047		00048	
Streptavidin	1 mg	29031	29032	29033	29034	29043	29038	29035	29036	29037	29041		29040		29042	
Wheat germ agglutinin	1 mg	29021-1			29022-1				29023-1	29024-1	29026-1				29025-1	
	5 x 1 mg	29021			29022				29023	29024	29026				29025	

CF™ Dye Bioconjugates Assay Kits

Table 2. Cell viability and apoptosis assay kits featuring CF™dye bioconjugates

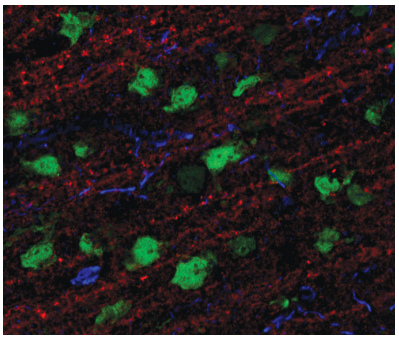
Product	Catalog number	Assays per kit	Description
CF™488 TUNEL Assay Apoptosis Detection Kit	30063	50	Stain the nuclei of apoptotic cells in fixed specimens with green fluorescence by TUNEL labeling of cleaved DNA with CF™488A-dUTP
CF™594 TUNEL Assay Apoptosis Detection Kit	30064	50	Stain the nuclei of apoptotic cells in fixed specimens with red fluorescence by TUNEL labeling of cleaved DNA with CF™594-dUTP
Apoptosis & Necrosis Quantitation Kit Plus	30065	50	Stain phosphatidylserine on the surface of apoptotic cells with CF™488A Annexin V, and detecting cells with compromised membrane integrity with the red fluorescent vital dye ethidium homodimer III.
Apoptotic, Necrotic & Healthy Cells Quantification Kit Plus	30066	50	Stain phosphatidylserine on the surface of apoptotic cells with CF™488A Annexin V, and detecting cells with compromised membrane integrity with the red fluorescent vital dye ethidium homodimer III. Includes the blue fluorescent DNA dye Hoechst 33342 to stain all cell nuclei.
Dual Apoptosis Assay Kit with NucView™ 488 Caspase-3 Substrate and CF™594 Annexin V	30067	50	Measure caspase-3 activity in live cells with the novel green fluorescent caspase-3 substrate NucView™488 and phosphatidylserine exposure with CF™594 Annexin V.
CF™488A Annexin V and 7-AAD Apoptosis Kit	30060	100	Measure phosphatidylserine exposure with CF™488A Annexin V to quantitate apoptotic cells, and stain cell with compromised membrane integrity with the red fluorescent vital dye 7-AAD.
CF™488A Annexin V and PI Apoptosis Kit	30061	100	Measure phosphatidylserine exposure with CF™488A Annexin V to quantitate apoptotic cells, and stain cell with compromised membrane integrity with the red fluorescent vital dye propidium iodide (PI).
Live Bacterial Gram Stain Kit	32000-1	200	Stain gram-positive bacteria with CF™594 wheat germ agglutinin (WGA). Includes the blue fluorescent DNA dye DAPI to stain gram-positive and gram-negative bacteria.
	32000	800	
Bacterial Viability and Gram Stain Kit	32001	200	Stain gram-positive bacteria with CF™488A wheat germ agglutinin (WGA), and dead bacteria with the red fluorescent vital dye ethidium homodimer III. Includes the blue fluorescent DNA dye DAPI to stain gram-positive and gram-negative bacteria.

CF™ Dye Antibody Conjugates

Whole IgG

Please see the following pages for information on Biotium's broad selection of CF™ dye-labeled secondary antibodies and F(ab')$_2$ fragments, isotype-specific anti-mouse antibodies, highly cross-absorbed secondary antibodies for multi-color labeling, and antibodies against biotin, GFP, and epitope tags.

Figure 1. Frozen section of rat cerebral cortex stained with mouse anti-MAP2B and CF633 donkey anti-mouse IgG, highly cross-adsorbed (red), rabbit anti-GFAP and CF555 donkey anti-rabbit IgG, highly cross-adsorbed (represented in blue), and CF488A-labeled anti-NeuN antibody (green).



Secondary antibodies, whole IgG (H+L)

2 mg/mL in PBS pH 7.4 containing 50% glycerol, 2 mg/ml BSA (IgG-free and protease-free) and 0.05% sodium azide

		CF™Dye/catalog number															
Antibody	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF750	CF770
Chicken Anti-Goat	0.5 mL				20225				20226	20227							
	50 uL				20225-1				20226-1	20227-1							
Chicken Anti-Mouse	0.5 mL				20208				20221	20222							
	50 uL				20208-1				20221-1	20222-1							
Chicken Anti-Rabbit	0.5 mL				20209				20223	20224							
	50 uL				20209-1				20223-1	20224-1							
Goat Anti-Guinea Pig	0.5 mL	20198			20017	20317	20036	20108	20118	20129	20085	20041					
	50 uL	20198-1			20017-1	20317-1	20036-1	20108-1	20118-1	20129-1	20085-1	20041-1					
Goat Anti-Mouse	0.5 mL	20140	20080	20180	20010	20306	20030	20100	20110	20120	20197	20040	20050	20054		20070	
	50 uL	20140-1	20080-1	20180-1	20010-1	20306-1	20030-1	20100-1	20110-1	20120-1	20197-1	20040-1	20050-1	20054-1		20070-1	
Goat Anti-Rabbit	0.5 mL	20141	20082	20181-1	20012	20309	20033	20102	20112	20122	20202	20043	20053	20055		20073	
	50 uL	20141-1	20082-1	20181	20012-1	20309-1	20033-1	20102-1	20112-1	20122-1	20202-1	20043-1	20053-1	20055-1		20073-1	
Goat Anti-Swine	0.5 mL				20028	20324	20236	20091	20160	20138	20089	20286					
	50 uL				20028-1	20324-1	20236-1	20091-1	20160-1	20138-1	20089-1	20286-1					
Rabbit Anti-Chicken	0.5 mL				20079	20312			20164	20165							
	50 uL				20079-1	20312-1			20164-1	20165-1							
Rabbit Anti-Goat	0.5 mL				20021	20315	20031	20107	20117	20128	20090	20049			20068		
	50 uL				20021-1	20315-1	20031-1	20107-1	20117-1	20128-1	20090-1	20049-1			20068-1		
Rabbit Anti-Guinea Pig	0.5 mL														20243		20244
	50 uL														20243-1		20244-1

Biotium regularly adds CF™ dye-conjugated secondary antibodies to our product line according to customer demand. Please check our website at www.biotium.com for updates, and contact us to request antibody/dye combinations above that do not have a catalog number. If you are looking for an antibody conjugate not listed in our catalog, please let us know. We may be able to add it as a new product, or perform a custom conjugation for you.

CF™ Dye Antibody Conjugates

F(ab')$_2$ fragments; isotype specific; anti-biotin, -GFP and -epitope tag

Secondary antibodies, F(ab')$_2$ fragments

2 mg/mL in PBS pH 7.4 containing 50% glycerol, 2 mg/ml BSA (IgG-free and protease-free) and 0.05% sodium azide

		CF™Dye/catalog number																
Antibody	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770
Goat Anti-Mouse	0.25 mL	20145			20011	20329	20032	20109	20119	20130	20086	20042			20063			
Goat Anti-Rabbit	0.25 mL	20146			20013	20330	20035	20099	20153	20131	20087	20045			20064			

Goat anti-mouse isotype-specific secondary antibody conjugates

Highly cross-adsorbed for multiple labeling (min X Bv, Hu, Rb)

2 mg/mL in PBS pH 7.4 containing 50% glycerol, 2 mg/ml BSA (IgG-free and protease-free) and 0.05% sodium azide

		CF™Dye/catalog number																
Isotype	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770
Goat Anti-Mouse IgG1	0.25 mL	20245			20246	20325	20247	20248	20249	20250	20251	20252			20253			20254
	0.125 mL	20245-2			20246-2	20325-2	20247-2	20248-2	20249-2	20250-2	20251-2	20252-2			20253-2			20254-2
	50 uL	20245-1			20246-1	20325-1	20247-1	20248-1	20249-1	20250-1	20251-1	20252-1			20253-1			20254-1
Goat Anti-Mouse IgG2a	0.25 mL	20255			20256	20326	20257	20258	20259	20260	20261	20262			20263			20264
	0.125 mL	20255-2			20256-2	20326-2	20257-2	20258-2	20259-2	20260-2	20261-2	20262-2			20263-2			20264-2
	50 uL	20255-1			20256-1	20326-1	20257-1	20258-1	20259-1	20260-1	20261-1	20262-1			20263-1			20264-1
Goat Anti-Mouse IgG2b	0.25 mL	20265			20266	20327	20267	20268	20269	20270	20271	20272			20273			20274
	0.125 mL	20265-2			20266-2	20327-2	20267-2	20268-2	20269-2	20270-2	20271-2	20272-2			20273-2			20274-2
	50 uL	20265-1			20266-1	20327-1	20267-1	20268-1	20269-1	20270-1	20271-1	20272-1			20273-1			20274-1

Anti-GFP, anti-hapten, and anti-epitope tag antibody conjugates

In PBS pH 7.4 containing 50% glycerol, 2 mg/ml BSA (IgG-free and protease-free) and 0.05% sodium azide

		CF™Dye/catalog number																
Antibody	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770
Mouse Monoclonal Anti-GFP, 1 mg/mL	0.1 mL				20215				20216	20217	20218				20219			20220
Mouse Monoclonal Anti-Biotin, 2 mg/mL	0.25 mL		20203		20204				20205	20206	20207							
	50 uL		20203-1		20204-1				20205-1	20206-1	20207-1							
Mouse Monoclonal Anti-Fluorescein, 2 mg/mL	0.25 mL			20214	20210				20211	20212	20213							
	50 uL			20214-1	20210-1				20211-1	20212-1	20213-1							
Rabbit Anti-HA tag, 1 mg/mL	50 uL				20238				20239		20240							
Mouse Monoclonal Anti-6X His, 1 mg/mL	50 uL				20228				20229		20237							

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CF™ Dye Antibody Conjugates

Highly cross-adsorbed for multiple labeling

Secondary antibodies, whole IgG (H + L) , highly cross-adsorbed for multiple-labeling

2 mg/mL in PBS pH 7.4 containing 50% glycerol, 2 mg/ml BSA (IgG-free and protease-free) and 0.05% sodium azide

			CF™Dye/catalog number																
Antibody	Min X React	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770
Bovine Anti-Goat	Bv, Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm	0.5 mL				20293	20313		20294	20295	20296	20297							
		0.25 mL																	
		50 uL				20293-1	20313-1		20294-1	20295-1	20296-1	20297-1							
Donkey Anti-Chicken	Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm	0.5 mL	20275			20166	20310			20167	20168								
		0.25 mL																	
		50 uL	20275-1			20166-1	20310-1			20167-1	20168-1								
Donkey Anti-Goat	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm	0.5 mL	20142			20016	20314	20039	20106	20116	20127	20179	20048	20051					
		0.25 mL														20060	20196		20277
		50 uL	20142-1			20016-1	20314-1	20039-1	20106-1	20116-1	20127-1	20179-1	20048-1	20051-1		20060-1	20196-1		20277-1
Donkey Anti-Guinea Pig	Bv, Ch, Gt, Hs, Hu, Ms, Rb, Sh, SHm	0.5 mL				20169	20316	20276		20170	20171								
		0.25 mL														20241			20242
		50 uL				20169-1	20316-1	20276-1		20170-1	20171-1					20241-1			20242-1
Donkey Anti-Human	Bv, Ch, GP, Gt, Hs, Ms, Rb, Rt, Sh, SHm	0.5 mL				20074	20318			20075	20076								
		0.25 mL														20278			20279
		50 uL				20074-1	20318-1			20075-1	20076-1					20278-1			20279-1
Donkey Anti-Mouse	Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm	0.5 mL	20350			20014	20305	20037	20105	20115	20124	20177	20046						
		0.25 mL															20194		
		50 uL	20350-1			20014-1	20305-1	20037-1	20105-1	20115-1	20124-1	20177-1	20046-1				20194-1		
Donkey Anti-Rabbit	Bv, Ch, Gt, GP, Hs, Hu, Ms, Sh, SHm	0.5 mL	20351			20015	20308	20038	20098	20152	20125	20178	20047						
		0.25 mL															20195	20298	
		50 uL	20351-1			20015-1	20308-1	20038-1	20098-1	20152-1	20125-1	20178-1	20047-1				20195-1	20298-1	
Donkey Anti-Rat	Bv, Ch, GP, Gt, Hs, Hu, Ms, Rb, Sh, SHm	0.5 mL				20027	20320		20092	20159	20137	20199							
		0.25 mL																	
		50 uL				20027-1	20320-1		20092-1	20159-1	20137-1	20199-1							
Donkey Anti-Sheep	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm	0.5 mL	20148			20024	20322	20234	20095	20156	20134	20083	20284			20062			
		0.25 mL																	
		50 uL	20148-1			20024-1	20322-1	20234-1	20095-1	20156-1	20134-1	20083-1	20284-1			20062-1			

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

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Continued next page

CF™ Dye Antibody Conjugates

Highly cross-adsorbed for multiple labeling

Secondary antibodies, whole IgG (H + L) , highly cross-adsorbed for multiple-labeling

2 mg/mL in PBS pH 7.4 containing 50% glycerol, 2 mg/ml BSA (IgG-free and protease-free) and 0.05% sodium azide

			CF™Dye/catalog number																
Antibody	Min X React	Unit Size	CF350	CF405S	CF405M	CF488A	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770
Goat Anti-Chicken	Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm	0.5 mL				20020	20311	20034	20104	20114	20126	20084	20044						
		0.25 mL																	
		50 uL				20020-1	20311-1	20034-1	20104-1	20114-1	20126-1	20084-1	20044-1						
Goat Anti-Human	Bv, Hs, Ms	0.5 mL				20022	20319	20230	20097	20154	20132	20081	20280						
		0.25 mL														20287			20288
		50 uL				20022-1	20319-1	20230-1	20097-1	20154-1	20132-1	20081-1	20280-1			20287-1			20288-1
Goat Anti-Mouse	Bv, Hs, Hu, Rb, Sw	0.5 mL	20143		20182-1	20018	20299	20231	20101	20111	20121	20175	20281	20052					
		0.25 mL														20065	20192		20077
		50 uL	20143-1		20182	20018-1	20299-1	20231-1	20101-1	20111-1	20121-1	20175-1	20281-1	20052-1		20065-1	20192-1		20077-1
Goat Anti-Mouse (**min X Rat**)	Bv, Ch, Gt, GP Hs Hu Rb **Rt**, Sh, SHm	0.5 mL				20302	20328		20301	20303		20304							
		0.25 mL																	
		50 uL				20302-1	20328-1		20301-1	20303-1		20304-1							
Goat Anti-Rabbit	Hu, Ms, Rt	0.5 mL	20144			20019	20300	20232	20103	20113	20123	20176	20282						
		0.25 mL														20067	20193		20078
		50 uL	20144-1			20019-1	20300-1	20232-1	20103-1	20113-1	20123-1	20176-1	20282-1			20067-1	20193-1		20078-1
Goat Anti-Rat	Bv, Hs, Hu, Rb	0.5 mL	20147			20023	20321	20233	20096	20155	20133	20088	20283						
		0.25 mL														20069			
		50 uL	20147-1			20023-1	20321-1	20233-1	20096-1	20155-1	20133-1	20088-1	20283-1			20069-1			
Rabbit Anti-Human	Ms	0.5 mL				20071				20072	20066								
		0.25 mL														20243			20244
		50 uL				20071-1				20072-1	20066-1					20243-1			20244-1
Rabbit Anti-Mouse	Hu	0.5 mL	20149			20026	20307	20235	20093	20158	20136	20200	20285						
		0.25 mL	20149-1													20061			
		50 uL				20026-1	20307-1	20235-1	20093-1	20158-1	20136-1	20200-1	20285-1			20061-1			
Rabbit Anti-Rat	Hu	0.5 mL				20025			20094	20157	20135	20201							
		0.25 mL																	
		50 uL				20025-1			20094-1	20157-1	20135-1	20201-1							
Rabbit Anti-Sheep	Hu	0.5 mL				20172	20323			20173	20174								
		0.25 mL																	
		50 uL				20172-1	20323-1			20173-1	20174-1								

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

CF™ Dye Related Products

EverBrite™ mounting medium is an antifade mounting medium uniquely formulated for preserving fluorescence of CF™ dyes and other fluorescent dyes across the entire visible and near-IR spectra. Unlike Vectashield®, EverBrite™ mounting media is compatible with cyanine-based fluorophores (e.g., Cy™2, Cy™5, Alexa Fluor® 647 and DyLight™ 649). EverBrite™ mounting media is available with DAPI, which eliminates the need for a separate nuclear counterstaining step. After mounting, slides can be immediately viewed or sealed and stored at 4°C for at least several months.

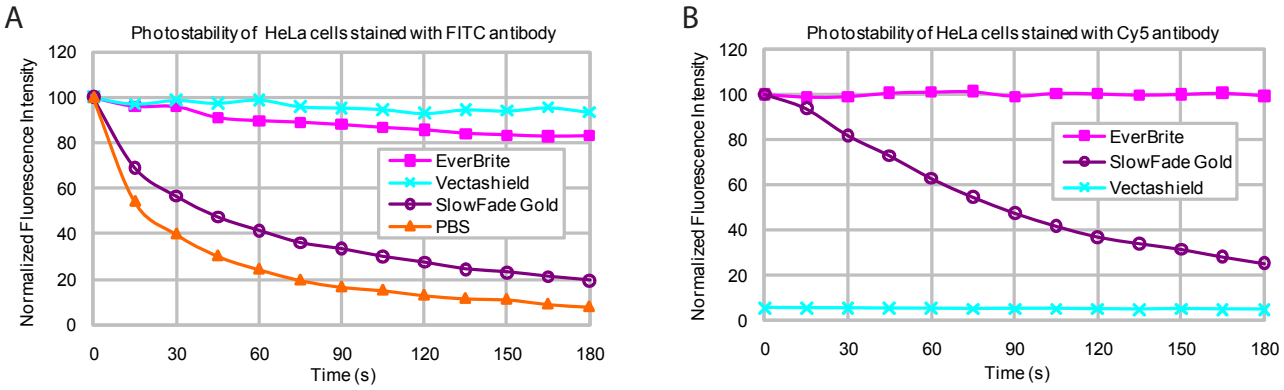

Figure 1. Intracellular staining of HeLa cells was performed using anti-tubulin antibody followed by goat anti-mouse-FITC (A) or goat anti-mouse-Cy5 (B). Cells were continuously exposed using a mercury arc lamp microscope. Images were captured every 15 seconds and fluorescence intensity of each frame was normalized to time 0. In B, data for Vectashield is not normalized in order to illustrate the intensity drop of Cy™5 dye.

CoverGrip™ Coverslip Sealant is an organic adhesive medium formulated specifically for sealing the edges of coverslips mounted using wet set antifade mounting medium for immunofluorescence. Traditionally, nail polish has been used to seal the edges of coverslips prior to fluorescence microscopy. However, components in nail polish such as isopropyl alcohol can leach into wet set mounting medium and interfere with specimen fluorescence, especially in the case of GFP. CoverGrip™ Coverslip Sealant is not miscible with aqueous mounting media, and dries to form clear, hard seal along the edges of the coverslip. CoverGrip™ Coverslip Sealant is made with limonene, a pleasant-smelling natural hydrocarbon made from citrus peels that is more environmentally friendly and less toxic than organic solvents. Available in 15 mL brush cap applicator bottles and 100 mL dropper-top refill bottles.

Cat.#	Product Name	Size
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23005	CoverGrip™ Coverslip Sealant, brush cap applicator bottle	15 mL
23005-1	CoverGrip™ Coverslip Sealant, refill	100 mL

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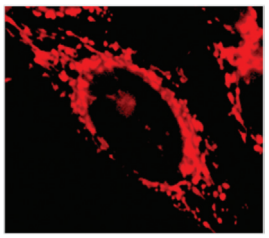
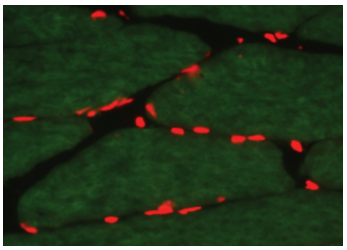
- JC-1 and other classic mitochondrial dyes
- Resazurin, MTT, and XTT viability assays
- Calcein-AM viability assays
- ATP-Glo™ Bioluminometric Cell Viability Assay Kit
- MCB glutathione detection kit



MitoView™ 633 mitochondrial membrane potential dye

Nuclear counterstains and vital dyes:

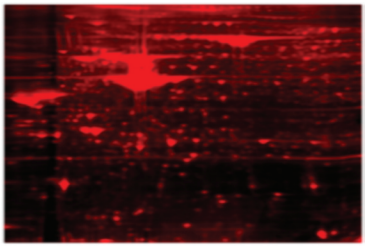
- RedDot™1 and RedDot™2 novel far red nucleus-specific stains for live and fixed cells
- DAPI and Hoechst blue fluorescent nuclear counterstains
- Ethidium homodimer III, propidium iodide, and other vital dyes



RedDot™2 far red nuclear counterstain

Other cell biology research tools:

- Carbocyanine lipophilic dye membrane labeling kits
- Cytosolic tracers
- Firefly and Renilla Luciferase Assay kits
- Enzyme substrates
- Fluorescent reagents for imaging calcium, pH, and other ions
- Biochemical reagents for nitric oxide detection



Lumitein™ protein gel stain

Genomics and proteomics products:

- GelRed™ and GelGreen™ nucleic acid gel stains: safer alternatives to ethidium bromide and SYBR™ stains
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- Lumitein™ fluorescent protein gel stain for one step staining of unfixed SDS-PAGE gels

Microbiology research tools

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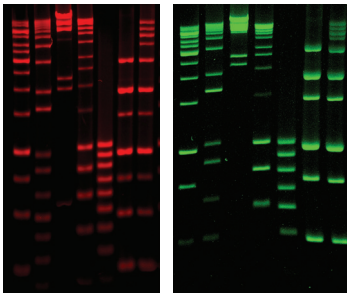

GelRed™ and GelGreen™ nucleic acid gel stains

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