# CF™ Dyes

## Next Generation Fluorescent Dyes


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Biotium

# CF™ Dyes Quick Reference Table

Visible spectrum

Far-red

Near-infrared

| CF™ dye | Page | $\lambda_{Ex}$ (nm) | $\lambda_{Em}$ (nm) | Excitation * | Replacement for | Features and applications |
|---|---|---|---|---|---|---|
| CF™350 | 5 | 347 | 448 | UV | Alexa Fluor® 350, AMCA, DyLight® 350 | • Brightest blue fluorescent conjugates for 350 nm excitation<br>• Highly water-soluble and pH insensitive |
| CF™405S | 5 | 404 | 431 | 405 nm | Alexa Fluor® 405, Cascade Blue®, DyLight® 405 | • Better compatibility with common instruments |
| CF™405M | 5 | 408 | 452 | 405 nm | BD Horizon™ V450, eFluor® 450, Pacific Blue® | • More photostable than Pacific Blue® dye with less green spill-over<br>• 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<br>• 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 | • Green dye with unique spectral properties |
| CF™488A | 7 | 490 | 515 | 488 nm | ATTO 488, Alexa Fluor® 488, Cy®2, DyLight® 488, FAM, FITC, Fluorescein | • Less non-specific binding and less red spill-over than Alexa Fluor® 488<br>• Very photostable<br>• Compatible with super-resolution imaging by TIRF** |
| CF™514 | 8 | 516 | 548 | 488 nm | Alexa Fluor® 514 | • Green dye that can be separated from CF™488A 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 super-resolution microscopy** |
| CF™543 | 10 | 541 | 560 | 532, 543, or 546 nm | Alexa Fluor® 546, Tetramethylrhodamine (TAMRA) | • Significantly brighter than Alexa Fluor® 546 |
| CF™555 | 10 | 555 | 565 | 532, 543, 546,, 555, or 568 nm | Alexa Fluor® 555, ATTO 550, Cy®3, DyLight® 549, TRITC | • Brighter than Cy®3<br>• Validated in multicolor super-resolution imaging by STORM** |
| CF™568 | 11 | 562 | 583 | 532, 543, 546, 555, or 568 nm | Alexa Fluor® 568, ATTO 565, Rhodamine Red | • Optimized for the 568 nm line of the Ar-Kr mixed-gas<br>• Brighter and more photostable than Alexa Fluor 568<br>• Compatible with TIRF and multicolor STORM** |
| CF™594 | 12 | 593 | 614 | 532, 543, 546, 555, or 568 nm | Alexa Fluor® 594, ATTO 594, DyLight® 594, Texas Red® | • Yields the brightest conjugates among spectrally similar dyes<br>• Extremely photostable |
| CF™594ST | 12 | 593 | 614 | 532, 543, 546, 555, or 568 nm | Unique dye for STORM** | • Specifically designed for super-resolution imaging by 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 | • Yields the brightest antibody conjugates among spectrally similar dyes<br>• Far more photostable than Alexa Fluor® 647<br>• Compatible with super-resolution TIRF, FIONA, and gSHRImP** |
| CF™640R | 15 | 642 | 662 | 633, 635, or 640 nm | Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649 | • Has the best photostability among dyes with Cy®5-like spectra<br>• Yields highly fluorescent protein conjugates<br>• Compatible with TIRF and FLIMP super-resolution techniques** |
| CF™647 | 16 | 650 | 665 | 633, 635, or 640 nm | Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649 | • Brighter than Cy®5<br>• Compatible with multicolor super-resolution imaging by STORM** |
| CF™660C | 17 | 667 | 685 | 633, 635, or 640 nm | Alexa Fluor® 660 | • Much brighter and more photostable than Alexa Fluor® 660<br>• Compatible with multicolor super-resolution imaging by STORM** |
| CF™660R | 17 | 663 | 682 | 633, 635, or 640 nm | Alexa Fluor® 660 | • Brighter than Alexa Fluor® 660<br>• 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<br>• Validated in multicolor STORM and dual-color 3D super-resolution imaging**<br>• 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<br>• Suitable for labeling nucleic acids and small biomolecules<br>• Compatible with LI-COR® Odyssey® System<br>• Compatible with STED and single molecule spectroscopy** |
| CF™750 | 19 | 755 | 777 | 680 or 685 nm | Alexa Fluor® 750, Cy®7, DyLight® 750, IRDye® 750 | • Exceptionally bright and stable<br>• Highly water soluble without bearing excessive charge<br>• Validated in super-resolution imaging by STORM** |
| CF™770 | 19 | 770 | 797 | 785 nm | DyLight® 800, IRDye® 800CW, ZW800-1 | • Exceptionally bright and stable<br>• Compatible with LI-COR® Odyssey® System |
| CF™790 | 19 | 784 | 806 | 785 nm | Alexa Fluor® 790 | • Exceptionally bright and stable<br>• Highly water soluble without bearing excessive charge |
| CF™800 | 19 | 797 | 816 | 785 nm | Spectrally similar to Indocyanine green | • Unique long wavelength near-infrared dye |

*Visible and far-red dyes can be excited by a UV light source for epifluorescence microscopy.

**See pp. 20-21 for more information about CF™ dyes for super-resolution microscopy

Alexa Fluor, Cascade Blue, Pacific Blue, and Texas Red are registered trademarks of Invitrogen; ATTO dyes are products of ATTO-TEC GmbH; BD Horizon is a trademark of BD Biosciences; Cy® is a registered trademark of GE Healthcare; DyLight is a registered trademark of Thermo Fisher Scientific; eFluor is a registered trademark of eBioscience; IRDye and Odyssey are registered trademarks of LI-COR Bioscience; Krome Orange is a trademark of Beckman Coulter; LightCycler is a registered trademark of Roche Applied Science.

# CF™ Dyes 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 the following 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 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.

## 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 (according to Life Technologies' 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.

## 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 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.

## 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 pp. 20-21 for more information.

## 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 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.

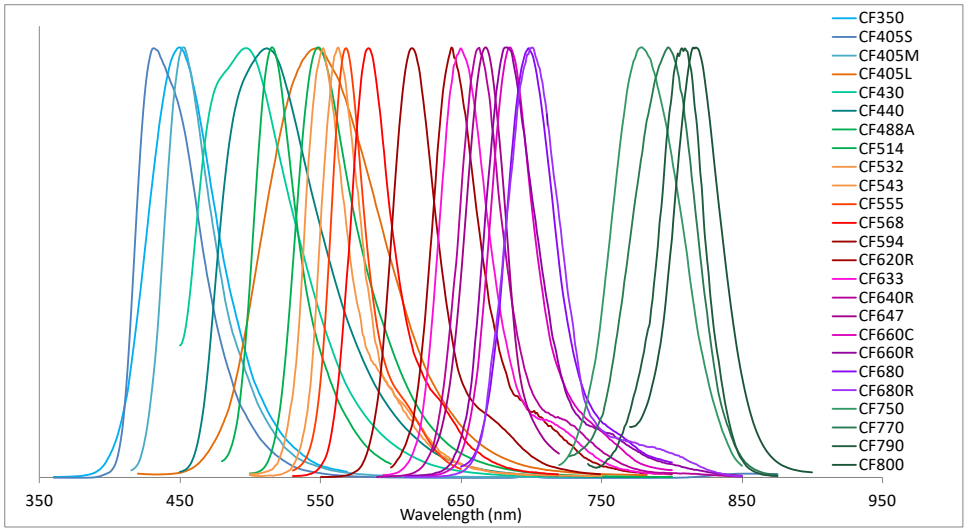
Biotium's near-IR CF™ dyes are based on the core structure of either cyanine dyes or rhodamine dyes. Those core structures are modified such that the intramolecular mobility of the dyes is restricted, which leads to higher quantum yield and better water solubility without adding excessive charge. As 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.

## Multi-color flexibility

Biotium currently offers 26 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™ dyes and Mix-n-Stain antibody labeling technology are covered by pending U.S. and international patents. We welcome inquiries about licensing the use of our dyes, trademarks or technologies; email us at btinfo@biotium.com.

**Emission spectra of CF™ dyes**




Alexa Fluor® is a registered trademark of Invitrogen; DyLight® is a registered trademark of Thermo Fisher Scientific; IRDye® is a registered trademark of Li-COR Bioscience.

# CF™ Dyes
## *Frequently Asked Questions*

| Question | Answer |
|---|---|
| What does the CF in CF dyes stand for? | CF initially was an abbreviation for Cyanine-based Fluorescent dyes. These were the first patented CF dyes based on cyanine dye structures. Since then, our CF dye patent portfolio has expanded to include four different fluorescent dye core structures that cover the fluorescence spectrum from UV to NIR. |
| 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 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 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: 1) chemical stability of the dye core structure; 2) stability of the reactive group; and 3) photostability of the dye.<br>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.<br>Reactive CF dyes comprise a reactive group used in 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 commercial SE dyes. This is because CF SE dyes are derived from aliphatic carboxylic groups, which results in a more stable SE form, while other commercial SE dyes usually are derived from aromatic carboxylic acid groups that yield a less stable SE form.<br>Photostability 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 commercial 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 pH range of at least 2 –11. The fluorescence of most CF dyes is relatively insensitive to pH, except for that of CF405M, CF568, CF620R, and CF633. 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 CF405s, CF405M, and CF405L? | 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 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 pp. 5-6 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 a few cyanine-based near-IR CF dyes carry 3-4 negative charges. However, the more negatively charged CF dyes comprise unique structural features that shield the negative charges such that the biomolecules (such as antibodies) the dyes label do not lose specificity due to the excessive negative charges. |
| Can CF dyes be used for STORM? | Several CF dyes have been validated in super-resolution imaging by STORM, as well as other super-resolution techniques. Biotium also offers dyes specifically designed for STORM imaging. See pp. 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. |

# CF™350

## *A bright UV-excitable blue fluorescent dye*

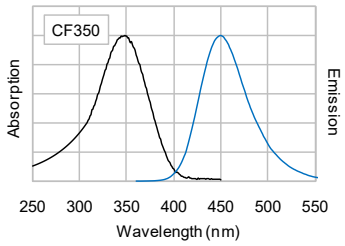
### Technical Summary

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

### Features

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

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



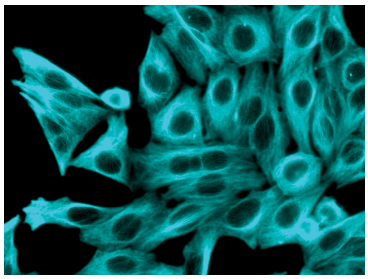



Figure 2. HeLa cells stained with mouse anti-tubulin antibody and CF350 goat anti-mouse IgG (cyan).



# 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

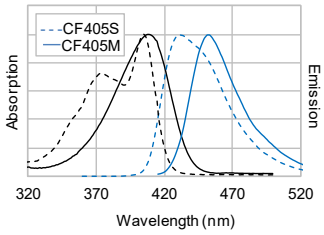
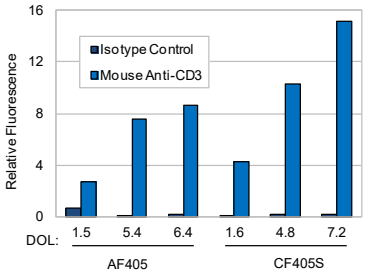



Figure 1. Absorption and emission spectra of CF405S and CF405M goat anti-mouse conjugates in PBS.

### Features

- CF™405S: Brighter than Alexa Fluor® 405
- CF™405M: More photostable than Pacific Blue®, with less spill-over in the green channel
- CF™405M: an excellent choice for super-resolution imaging by SIM (see pp. 20-21)

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.



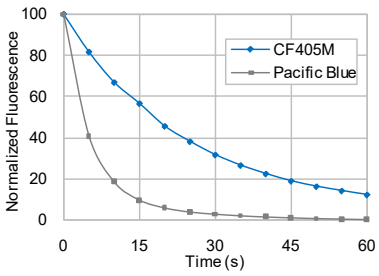




Figure 3. Photostability of CF405M and Pacific Blue. CF405M and Pacific Blue dye solutions were continuously exposed to mercury arc lamp microscope excitation with a DAPI filter set. Images were captured every 5 seconds for one minute. Fluorescence intensity was normalized to time 0.

CF™405L

# CF™405L Dye

*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®

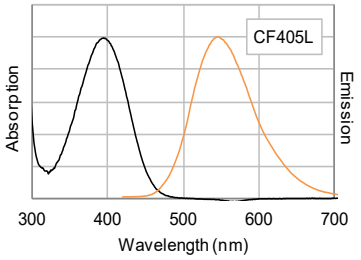



Figure 1. Absorption and emission spectra of CF405L goat anti-mouse conjugate in PBS.

CF™430 & CF™440

# 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
- CF430 is a perfect match for the CFP filter set
- Suitable for flow cytometry in the AmCyan channel
- Highly water soluble and pH-insensitive

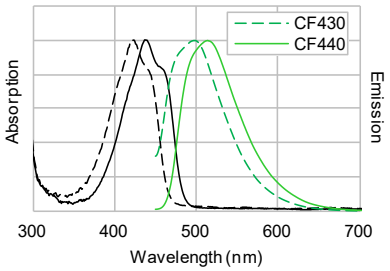



Figure 1. Absorption and emission spectra of CF430 and CF440 goat anti-mouse conjugates in PBS.

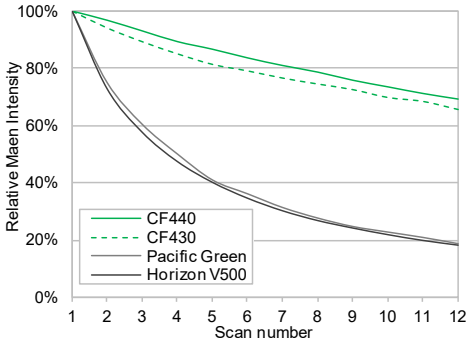



Figure 2. Relative photostability of CF430 and CF440 compared to spectrally-similar dyes. Cells were stained with biotinylated primary antibodies followed by streptavidin conjugates of CF430, CF440, Pacific Green, or BD Horizon V500. Fluorescence was imaged on a Zeiss LSM700 confocal microscope in the 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.

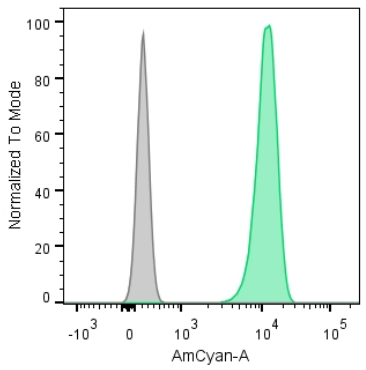



Figure 3. Flow cytometry analysis of Jurkat cells stained with isotype control (gray peak) or mouse anti-CD3 (green peak) followed by CF430 goat anti-mouse IgG, analyzed in the AmCyan channel of a BD LSRII flow cytometer.

# CF™450

## *A green fluorescent 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

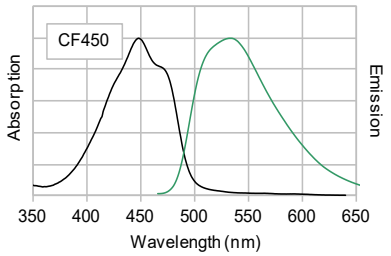



Figure 1. Absorption and emission of CF450 goat anti-mouse conjugate in PBS.



# 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 (aka FITC, FAM), Cy®2

### Features

- Minimally charged, for less non-specific binding than Alexa Fluor® 488
- Narrower emission spectrum for less bleed to red
- Very photostable
- Compatible with super-resolution imaging by TIRF (see pp. 20-21)
- Highly water soluble and pH-insensitive

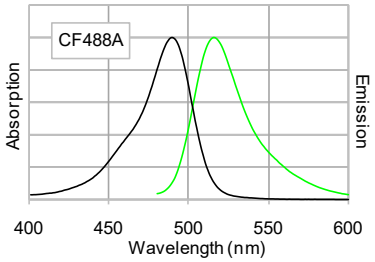



Figure 1. Absorption and emission spectra of CF488A goat anti-mouse conjugate in PBS.

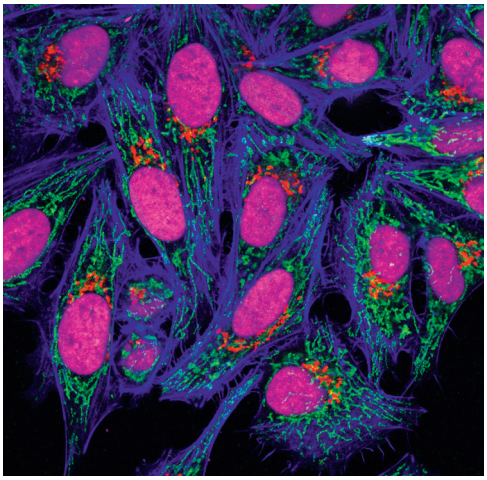


Figure 2. HeLa cells stained with rabbit anti-COXIV and CF488A goat anti-rabbit IgG (mitochondria, green) , mouse anti-Golgin 97 and CF™555 goat anti-mouse IgG (Golgi, red), CF405M phalloidin (actin filaments, blue), and RedDot2 (nuclei, magenta). See p. 30 for more information on RedDot2.

# CF™514

*Alternative green fluorescent dye*

## Technical Summary

Abs/Em Maxima: 516/548 nm

Extinction coefficient: 105,000

Molecular weight: ~1216

Excitation laser line: 488 nm

Replaces: Alexa Fluor® 514

## Features

- Image using the same settings as FITC or CF™488A
- Can be distinguished from CF™488A in the same specimen by spectral imaging and linear unmixing

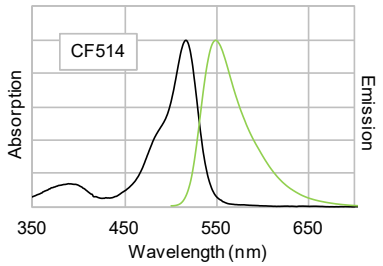



Figure 1. Absorption and emission spectra of CF514 goat anti-mouse conjugate in PBS.

# CF™532

*A bright orange fluorescent dye for the 532 nm laser*

CF™532

## Technical Summary

Abs/Em Maxima: 527/558 nm

Extinction coefficient: 96,000

Molecular weight: ~ 685

Excitation laser line: 532 nm

Direct replacement for: 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

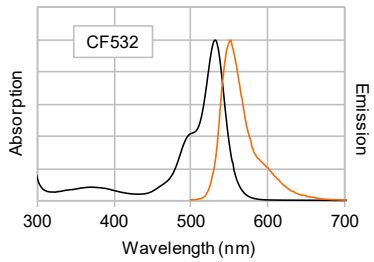



Figure 1. Absorption and emission spectra of CF532 goat anti-mouse IgG conjugate in PBS.

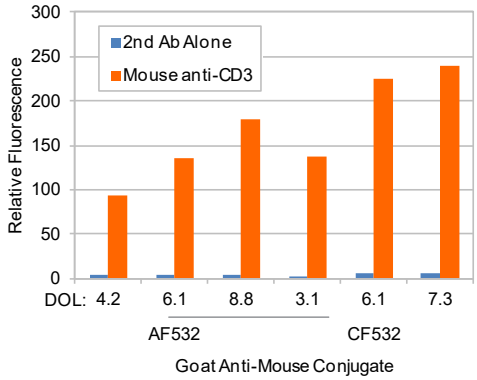



Figure 2. Flow cytometry analysis of Jurkat cells stained with Alexa Fluor 532 (AF532) antibody or CF532 secondary antibody conjugates. Intracellular staining was performed with mouse anti-CD3 antibody followed by goat anti-mouse IgG conjugates. Background was determined by staining with secondary antibody (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*

CF™535ST

## Technical Summary

Abs/Em Maxima: 535/568 nm

Extinction coefficient: 95,000

Molecular weight: ~728

Excitation laser line: 532 nm

See pp. 20-21 for more information about CF™ dyes for super-resolution imaging.

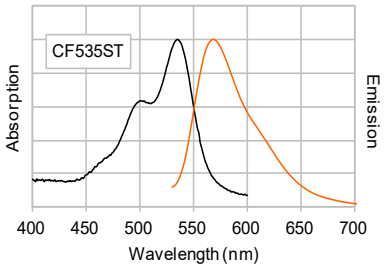



Figure 1. Absorption and emission spectra of CF535ST goat anti-mouse IgG conjugate in PBS.

CF™543

# CF™543

## *An orange fluorescent 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 nm, 543 nm, or 546 nm

Direct replacement for: Alexa Fluor® 546, TAMRA

### Features

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

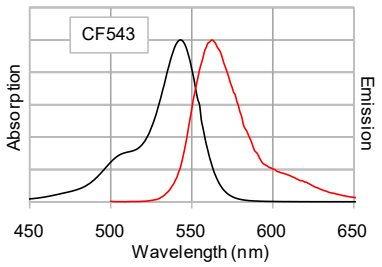



Figure 1. Absorption and emission spectra of CF543 goat anti-mouse conjugate in PBS.

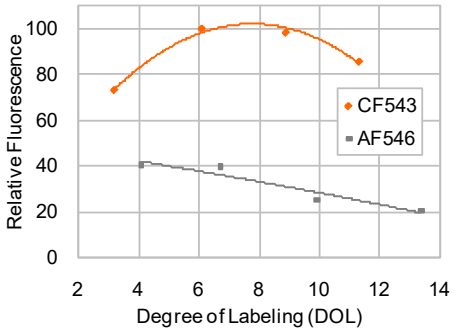



Figure 2. Relative fluorescence of CF543 and Alexa Fluor 546 (AF546) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

CF™532

# 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, ATTO 550, Cy®3, DyLight® 549, Rhodamine

### Features

- Brighter than Cy®3
- Highly water-soluble
- Validated in multicolor STORM super-resolution imaging (see pp. 20-21)

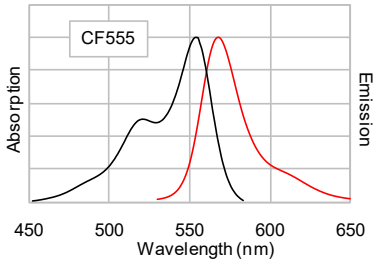



Figure 1. Absorption and emission spectra of CF555 goat anti-mouse conjugate in PBS.

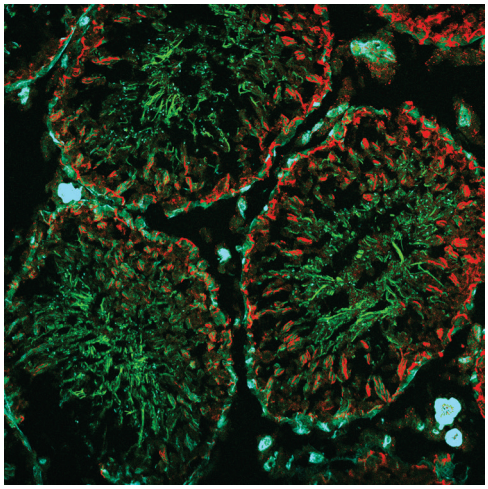


Figure 2. Frozen section of rat testis stained with mouse anti-tubulin and CF488A goat anti-mouse (min x rat) (microtubules, green), CF555 Mix-n-Stain labeled mouse anti-ZO1 (tight junctions, red) and CF640R phalloidin (actin filaments, cyan). See p. 23 for more information on Mix-n-Stain antibody labeling 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

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

### Features

- Yields much brighter antibody conjugates than Alexa Fluor® 568
- Extremely photostable
- Excellent choice for multi-color imaging with CF™488A and CF™640R
- Compatible with TIRF and multicolor STORM super-resolution imaging (see pp. 20-21)

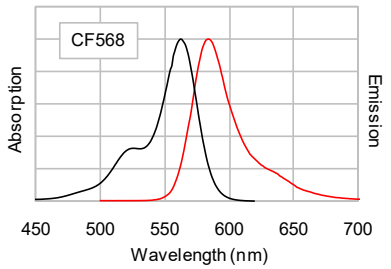



Figure 1. Absorption and emission spectra of CF568 goat anti-mouse conjugate in PBS.

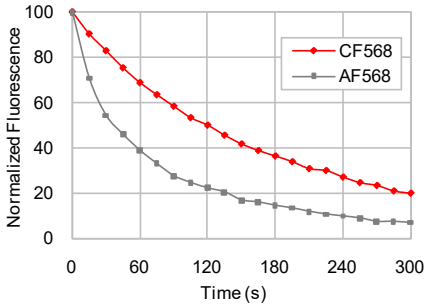



Figure 3. Photostability of CF568 and Alexa Fluor 568 (AF568) streptavidin conjugates. Intracellular staining of Jurkat cells was performed using anti-CD3-biotin followed by streptavidin-CF568 or streptavidin-AF568. Cells were continuously exposed to mercury arc lamp microscope excitation with a Cy3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

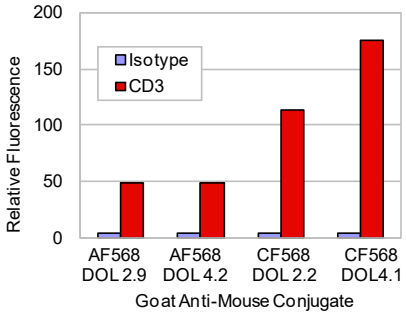



Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL2 channel. Bars represent the relative fluorescence of the geometric means of the cell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

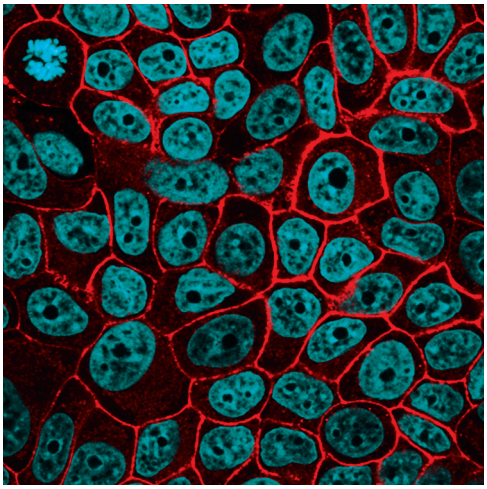


Figure 4. MCF-7 cells stained with CF568 monoclonal anti-Ep-CAM (clone EGP40/826) at 5 ug/mL (red). Nuclei are counterstained with Hoechst 33342 (blue). See p. 26 for more information on primary antibody conjugates.

# CF™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

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

## Features

- Yields the brightest antibody conjugates among spectrally similar dyes.
- Excellent choice for multicolor imaging with green dyes like CF™488A
- Extremely photostable
- Also see CF™594ST, a version of CF™594 engineered specifically for STORM microcopy (pp. 20-21).

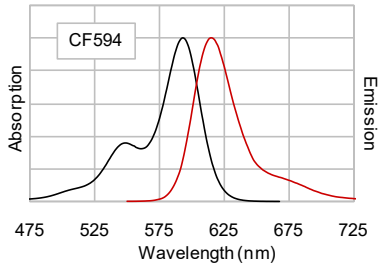



Figure 1. Absorption and emission spectra of CF594 goat anti-mouse conjugate in PBS.

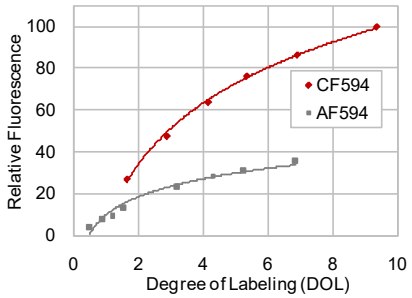



Figure 2. Relative fluorescence of CF594 and Alexa Fluor 594 (AF594) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

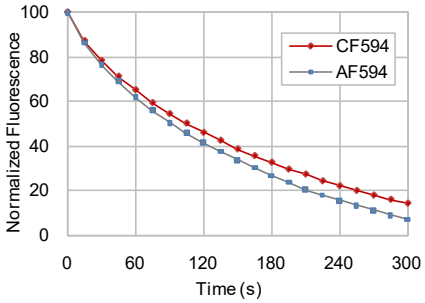



Figure 3. Photostability of CF594 and Alexa Fluor 594 (AF594) goat anti-mouse conjugates. Intracellular staining of Jurkat cells was performed with mouse anti-CD3 followed by CF594 or AF594 goat anti-mouse conjugates. Cells were continuously exposed to mercury arc lamp microscope excitation with a Texas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

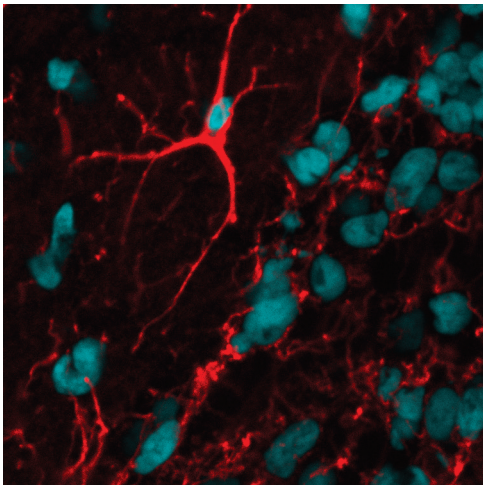


Figure 4. Glial cells in frozen section of rat brain stained with rabbit anti-GFAP antibody and CF594 goat anti-rabbit IgG (red). Nuclei are stained with RedDot2 (pseudocolored cyan). Mounted with Everbrite Mounting Medium. See p. 30 for more information on RedDot2 and EverBrite Mounting Medium.

# 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

Replaces: LightCycler® Red 640

## Features

- Highly water-soluble
- Highly fluorescent and extremely photostable
- Absorption/emission at 617/639 nm for use in FRET or multi-color detection

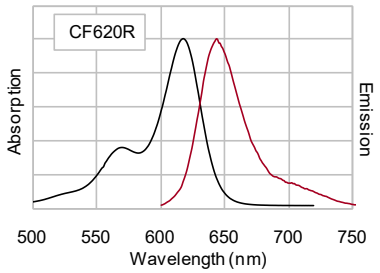



Figure 1. Absorption and emission spectra of CF620R free acid in PBS.

# CF™633

*The best dye for 633/635 laser lines*

## Technical Summary

Abs/Em Maxima: 630/650 nm

Extinction coefficient: 100,000

Molecular weight: ~820

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 pp. 20-21)

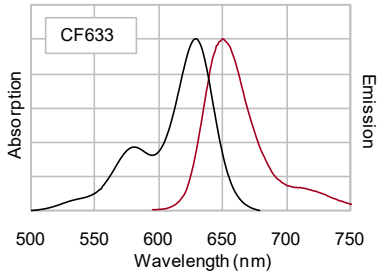



Figure 1. Absorption and emission spectra of CF633 goat anti-mouse conjugate in PBS.

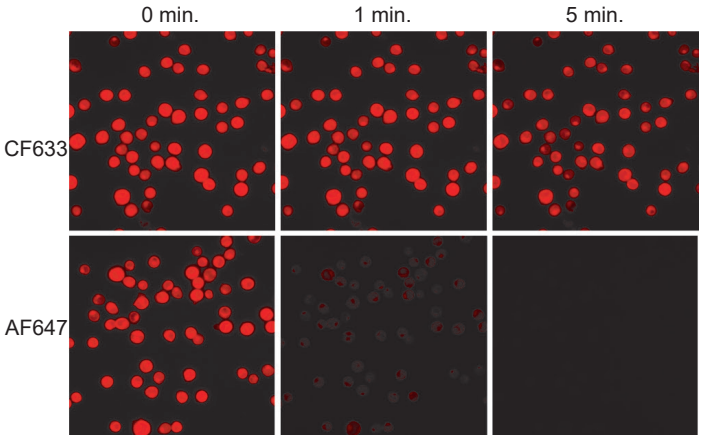



Figure 3. Relative photostability of CF633 and Alexa Fluor 647 (AF647) goat anti-mouse conjugates. Jurkat cells were fixed, permeabilized and stained with rabbit anti-CD3 followed by CF633 or Alexa Fluor 647 goat anti-rabbit IgG conjugates. Cells were imaged using a mercury arc lamp microscope equipped with a Cy5 filter set and CCD camera. Sequential images were captured at 0, 1, and 5 minutes.

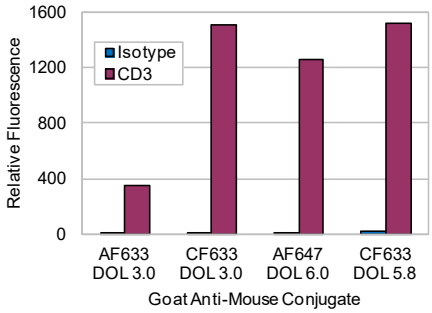



Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel. Bars represent the relative fluorescence of the geometric means of the cell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

# CF™640R

*A highly photostable far-red dye*



## 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

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 pp. 20-21)

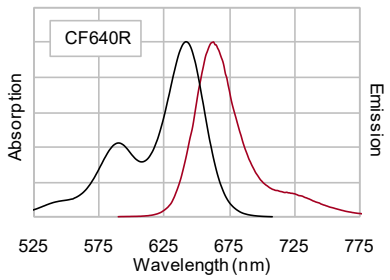



Figure 1. Absorption and emission spectra of CF640R goat anti-mouse conjugate in PBS.

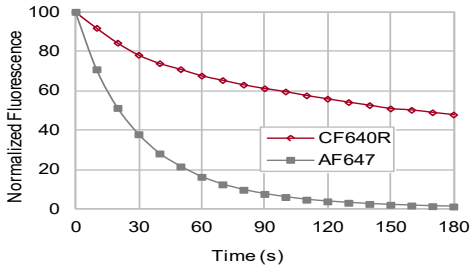



Figure 3. Photostability comparison between CF640R and Alexa Fluor 647 (AF647). HeLa cells were stained with anti-tubulin antibody conjugates of each dye. Cells were continuously illuminated by a mercury arc lamp microscope and sequential images were captured at 0, 1, and 3 minutes. Mean fluorescence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

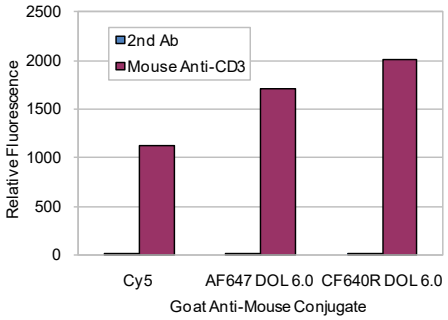



Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-CD3 or isotype control followed by goat anti-mouse IgG conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel. Bars represent the relative fluorescence of the geometric means of the cell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

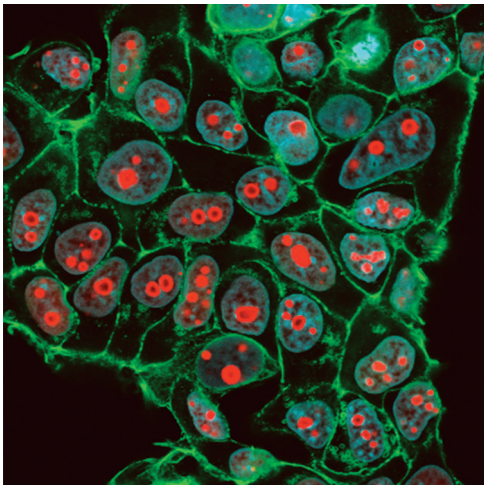


Figure 4. MCF-7 cells stained with CF640R monoclonal anti-Cyclin B1 (clone CCNB1/1098) at 5 ug/mL (red). Nuclei are counterstained with Hoechst 33342 (blue) and actin filaments are stained with CF488A phalloidin (green). See p. 26 for more information on primary antibody conjugates.

# 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 nm, 635 nm or 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 super-resolution imaging by STORM (see pp. 20-21)

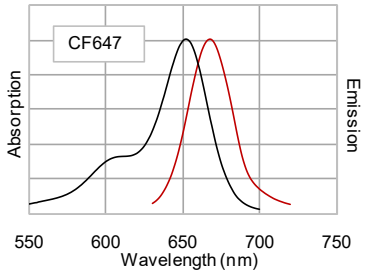



Figure 1. Absorption and emission spectra of CF647 goat anti-mouse conjugate in PBS.

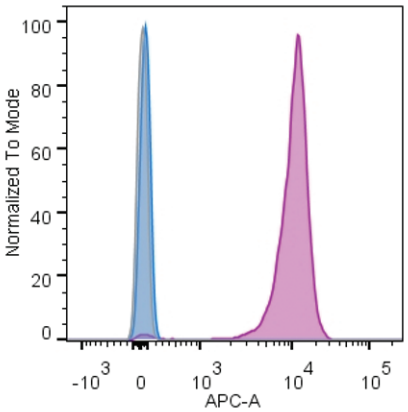



Figure 2. Intracellular staining of Jurkat cells with CF647 monoclonal anit-nucleollin (clone 365-2) (pink) or CF647 IgG1 isotype control (blue) at 1 ug/tube, compared to unstained cells (yellow). Cells were analyzed in the APC channel of a BD LSRII flow cytometer. See p. 26 for more information on primary antibody conjugates.

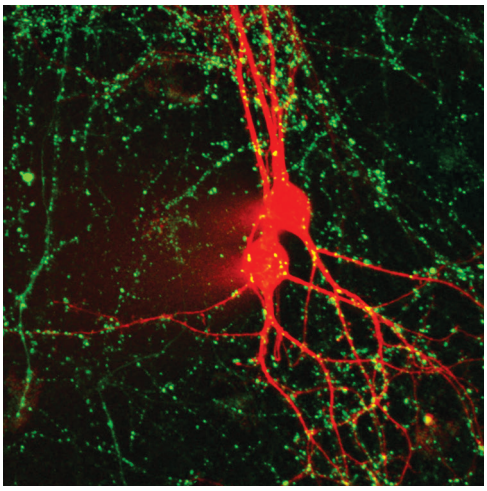


Figure 3. Cultured rat hippocampal neurons microinjected with CF647 hydrazide (red) and stained with SynaptoGreen™ C4 (FM1-43) (green, synaptic vesicles). Image courtesy of Professor Guosong Liu, Tsinghua University, Beijing, China.

# 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 nm, 635 nm or 640 nm

Replaces: 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

Replaces: Alexa Fluor® 660, Allophycocyanin (APC)

## CF™660C Features

- Much brighter and more photostable than Alexa Fluor® 660
- Compatible with multicolor super-resolution imaging by STORM (see pp. 20-21)

## CF™660R Features

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

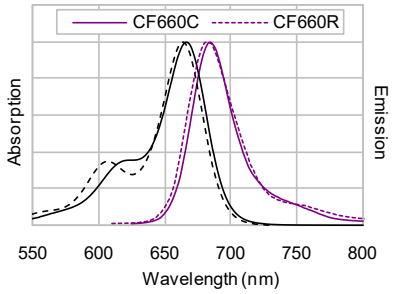



Figure 1. Absorption and emission spectra of CF660C and CF660R goat anti-mouse conjugates in PBS.

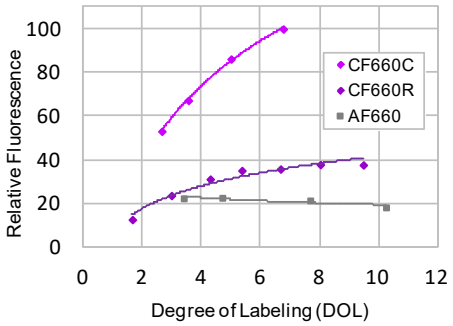



Figure 2. Relative fluorescence of CF660, CF660R and Alexa Fluor 660 (AF660) goat anti-mouse conjugates as a function of the number of dye molecules per protein (degree of labeling).

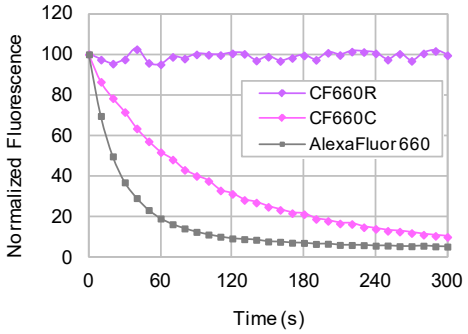



Figure 3. Photostability of CF660C, CF660R, and Alexa Fluor 660 (AF660) goat anti-mouse conjugates. HeLa cells were stained with mouse anti-tubulin followed by CF660C, CF660R or AF660 goat anti-mouse IgG conjugates. Cells were continuously exposed to mercury arc lamp microscope excitation using a Cy5 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*

## 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, 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

Replaces: Alexa Fluor® 680, Cy®5.5, IR®Dye 680

## CF™680 Features

- The brightest among spectrally similar dyes
- Validated in multicolor STORM and 3D super-resolution microscopy (see pp. 20-21)
- Compatible with LI-COR® Odyssey®

## CF™680R Features

- Unrivaled photostability among spectrally similar dyes
- Compatible with STED and single molecule spectroscopy super-resolution imaging (see pp. 20-21)
- Molecular weight compatible with nucleic acid labeling
- Compatible with LI-COR® Odyssey®

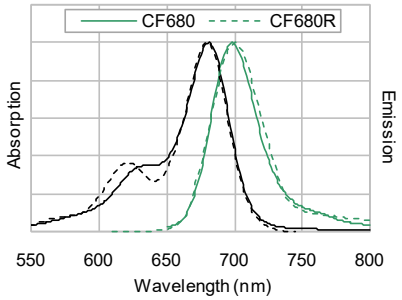



Figure 1. Absorption and emission spectra of CF680 and CF680R goat anti-mouse conjugates in PBS.

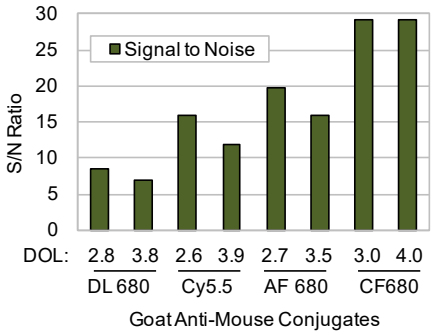



Figure 2. Intracellular staining of Jurkat cells was performed using mouse anti-human CD3 antibody or isotype control followed by goat anti-mouse secondary antibody conjugates. Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel Bars represent the signal-to-noise ratio of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

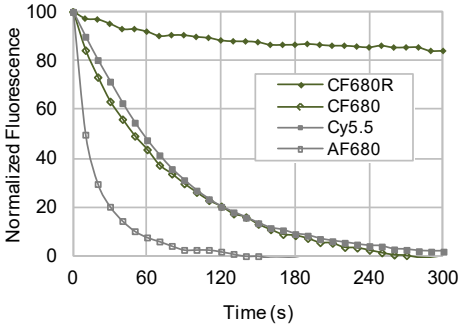



Figure 3. Photostability of far red dye conjugates. Jurkat cells were stained with mouse anti-CD3 followed by the indicated goat anti-mouse IgG conjugates. Cells were continuously exposed to mercury arc lamp excitation with a Cy5 filter set. Images were captured every 10 seconds for 5 minutes and fluorescence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

# CF™750, CF™770, CF™790, and CF™800

*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

Replaces: Alexa Fluor® 750, Cy®7, DyLight® 750

**CF™770**

Abs/Em Maxima: 770/797 nm

Extinction coefficient: 220,000

Molecular weight: ~ 3138

Excitation laser line: 785 nm

Replaces: DyLight™ 800, IRDye 800CW

**CF™790**

Abs/Em Maxima: 784/806 nm

Extinction coefficient: 210,000

Molecular weight: ~ 3267

Excitation laser line: 785 nm

Replaces: Alexa Fluor® 790

**CF™800**

Abs/Em Maxima: 797/816

Extinction coefficient: 210,000

Molecular weight: ~3334

Excitation laser line: 785 nm

Spectrally similar to: Indocyanine Green

### Features

- Exceptionally bright and stable
- Ideal for in vivo imaging
- Compatible with LI-COR® Odyssey®
- Superior signal-to-noise for bioconjugates
- CF™750 validated in STORM microscopy (see pp. 20-21)

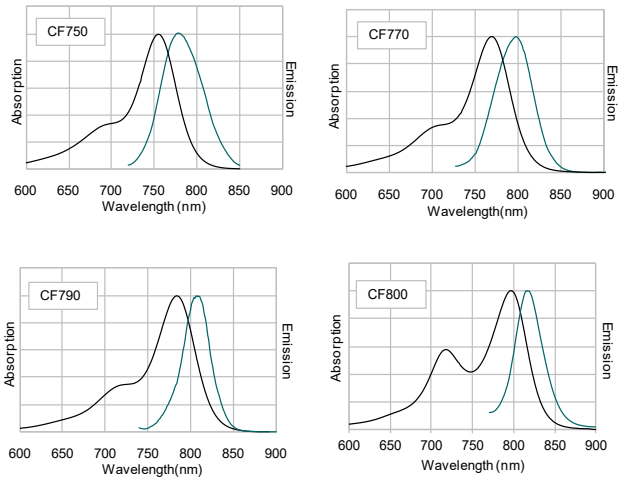



Figure 1. Absorption and emission spectra of near-IR CF dye goat anti-mouse conjugates in PBS.

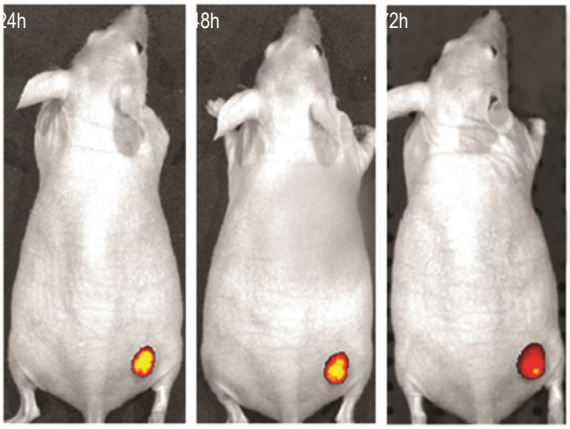



Figure 2. Tumors in mice were imaged using an IVIS® imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin conjugated to CF750. Images courtesy of Caliper Life Sciences.

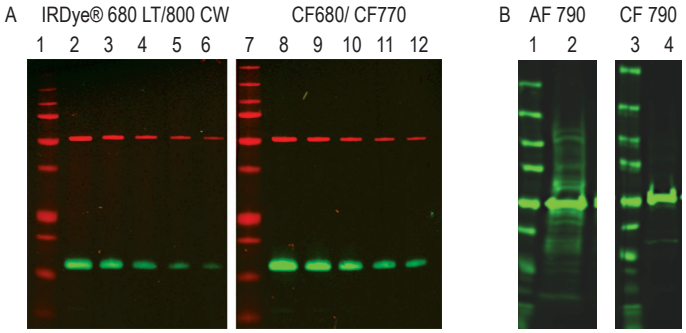



Figure 3. Near-infrared western blotting with CF dyes compared to spectrally similar dyes. A. Two-fold dilutions of HeLa cell lysate containing 2 ug, 1 ug, 0.5 ug, 0.25 ug. 0.125 ug total protein per lane were separated by SDS-PAGE, transferred to Immobilon FL PVDF (Millipore), and probed with mouse 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 CF680 goat anti-mouse (red) and CF770 goat anti-rabbit (green) (lanes 7-12) at the same final concentrations. Membranes were scanned using an Odyssey® infrared imaging system. Quantitation of the bands showed approximately 1.5-2-fold higher fluorescence intensity of CF dye secondary antibodies compared to IRDye secondary antibodies. Lanes 1 and 7 contain Odyssey Molecular Weight Marker (LI-COR Biosciences). 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 CF790 (right). CF790 does not introduce excessive negative charge to antibody conjugates, which can increase non-specific binding. Lanes 1 and 3 contain Dylight 680/800 Protein Ladder (Pierce).

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# 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. See Lehmann et al. 2015, and a full list of references for CF™ dye single-molecule imaging applications on page 21. Biotium offers a wide selection of CF™ dye labeled secondary antibodies, other conjugates, and labeling kits; visit www.biotium.com for our full selection of products.

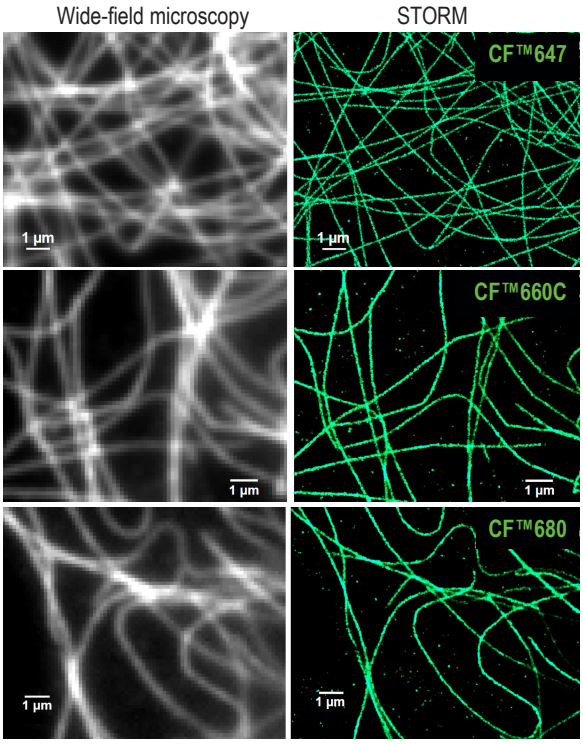


Figure 1. Comparison of conventional wide-field microscopy (left) with STORM (right) using CF™ dye conjugates. Fixed cells were stained with mouse anti-tubulin antibody followed by CF™ dye conjugated anti-mouse secondary antibody (top row: CF™647, middle row: CF™660C, bottom row: CF™680). For STORM, 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.

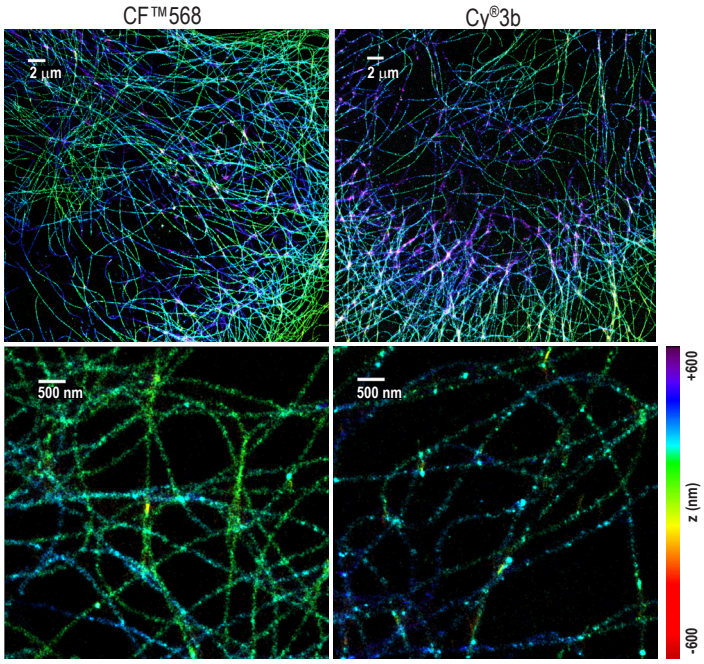


Figure 2. CF™568 (left) produces better images than Cy®3b (right) in 3-D STORM microscopy. Fixed cells were stained with mouse anti-tubulin antibody followed by dye-conjugated anti-mouse secondary antibodies. See Figure 1 legend for imaging conditions. Dye molecules were photoswitched and imaged using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

STORM microscopy images courtesy of Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

# CF™ Dyes for Super-Resolution Imaging



## Super-resolution imaging techniques validated for CF™ dyes

| CF™ Dye | Abs/Em maxima (nm) | Extinction coefficient | Super resolution application | References |
|---|---|---|---|---|
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| CF™488A | 490/515 | 70,000 | TIRF | Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200. |
| CF™535ST | 535/568 | 95,000 | STORM | Collaborator communication; contact techsupport@biotium.com for more information. |
| CF™555 | 555/565 | 150,000 | Multicolor STORM | Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119 |
| CF™568 | 562/583 | 100,000 | TIRF, multicolor STORM | Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119<br>Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200.<br>Zhang, M. et al. (2015). Translocation of interleukin-1β into a vesicle intermediate in autophagy-mediated secretion. eLife 2015;10.7554/eLife.11205 |
| CF™594ST | 593/614 | 115,000 | STORM | Collaborator communication; contact techsupport@biotium.com for more information. |
| CF™633 | 630/650 | 100,000 | TIRF, FIONA, gSHRImP | Bosch, P. J. et al. (2014). Evaluation of fluorophores to label SNAP-tag fused proteins for multicolor single-molecule tracking microscopy in live cells. Biophys J 107, 803-814.<br>Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200.<br>Kim, H. J., and Selvin, P. R. (2013). Fluorescence Imaging with One Nanometer Accuracy. SpringerReference Encyclopedia of Biophysics<br>Simonson, P. D.,Rothenberg, E., and Selvin, P. R. (2011). Single-molecule-based super-resolution images in the presence of multiple fluorophores. Nano Lett 11, 5090-5096. DOI:10.1021/nl203560r |
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| CF™750 | 755/777 | 250,000 | STORM | Collaborator communication; contact techsupport@biotium.com for more information. |

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

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CF™ dyes and conjugates have been cited in hundreds of publications, with new references published every day. Visit www.biotium.com/downloads for a more comprehensive list of CF™ dye references by color and application.


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- VivoBrite™ kits feature our superior near-infrared CF™ dyes for in vivo imaging

| | | | | Mix-n-Stain Antibody Labeling Kits | | | Mix-n-Stain Small Ligand Labeling Kits | |
|---|---|---|---|---|---|---|---|---|
| Label | Ex/Em (nm) | SE Protein Labeling Kits | VivoBrite Antibody Labeling Kits | 1 labeling of 5-20 ug antibody | 1 labeling of 20-50 ug antibody | 1 labeling of 50-100 ug antibody | For cell surface targets | For intracellular targets |
| Biotin | N/A | 92224 | | 92286 | 92266 | 92244 | | |
| FITC | 494/519 | | | 92294 | 92295 | 92296 | | |
| CF™350 | 347/448 | 92210 | | 92270 | 92250 | 92230 | | |
| CF™405S | 404/431 | 92211 | | 92271 | 92251 | 92231 | | |
| CF™405M | 408/452 | 92212 | | 92272 | 92252 | 92232 | 92362 | |
| CF™408 | 408/450 | | | | | | | 92356 |
| CF™405L | 395/495 | 92228 | | 92303 | 92304 | 92305 | | |
| CF™488A | 490/515 | 92213 | | 92273 | 92253 | 92233 | 92350 | |
| CF™500 | 500/510 | | | | | | | 92357 |
| CF™514 | 516/548 | | | 92231 | 92232 | 92233 | | |
| CF™532 | 527/558 | 92208 | | 92289 | 92290 | 92291 | | |
| CF™540 | 540/570 | | | | | | | 92358 |
| CF™543 | 541/560 | 92209 | | 92287 | 92267 | 92247 | | |
| CF™555 | 555/565 | 92214 | | 92274 | 92254 | 92234 | | 92364 |
| CF™568 | 562/583 | 92215 | | 92275 | 92255 | 92235 | 92351 | |
| CF™594 | 593/614 | 92216 | | 92276 | 92256 | 92236 | 92352 | |
| CF™633 | 630/650 | 92217 | | 92277 | 92257 | 92237 | 92353 | |
| CF™640R | 642/662 | 92225 | | 92278 | 92258 | 92245 | 92354 | |
| CF™647 | 650/665 | 92218 | | 92279 | 92259 | 92238 | 92359 | |
| CF™650 | 650/670 | | | | | | | 92363 |
| CF™660C | 667/685 | 92219 | | 92280 | 92260 | 92239 | 92360 | |
| CF™660R | 663/682 | 92223 | | 92281 | 92261 | 92243 | | |
| CF™680 | 681/698 | 92220 | 92160 | 92282 | 92262 | 92240 | 92361 | |
| CF™680R | 680/701 | 92226 | | 92283 | 92263 | 92246 | 92355 | |
| CF™750 | 755/777 | 92221 | 92161 | 92284 | 92264 | 92241 | | |
| CF™770 | 770/797 | 92222 | 92162 | 92285 | 92265 | 92242 | | |
| CF™790 | 784/806 | | 92163 | 92288 | 92268 | 92248 | | |
| R-PE | 496,564/578 | | | | 92298** | 92299 | | |
| APC | 650/660 | | | | 92306** | 92307 | | |
| Per-CP | 482/678 | | | | 92308** | 92309 | | |
| APC-CF750T | 650/780 | | | | 92310** | 92311 | | |
| HRP | N/A | | | 92300* | 92301** | 92302 | | |
| Alk. Phos. | N/A | | | | 92314** | 92315 | | |
| Glucose oxidase | N/A | | | | 92312** | 92313 | | |
| digoxigenin | | | | 92328 | 92329 | 92330 | | |
| DNP | | | | 92325 | 92326 | 92327 | | |

*10-20 ug labeling; **25-50 ug labeling

# CF™ Dye Reactive Dyes

*A wide selection of colors and functional groups for dye conjugation*

| Reactive group/ unit size | Alkyne 0.5 mg | Amine 1 mg | Aminooxy 1 mg | Azide 0.5 mg | BCN 0.5 mg | Hydrazide 1 mg | Maleimide 1 umol | MTS 1 mg | Picolyl azide 0.5 mg | SE 1 umol | Tyramide** 0.5 mg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reacts with | Azides, picolyl azides | Activated carboxylic acids | Aldehydes & ketones | Alkynes, BCN | Azides | Polar tracer* | Thiols | Thiols | Alkynes | Primary amines;lysine residues | HRP substrate |
| 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 | |
| CF™405L | | | | | | | 92046 | | | 92112 | 92198 |
| CF™430 | | | | | | | 92118 | | | 92117 | |
| CF™440 | | | | | | | 92124 | | | 92123 | |
| CF™450 | | | | | | | 96012 | | | 96011 | |
| CF™488A | 92086 | 92037 | 92051 | 92080 | 92075 | 92152 | 92022 | 92097 | 92187 | 92120 | 92171 |
| CF™500 | | | | | 96026 | | | | | | |
| CF™514 | | | | | | | | | | 92103 | 92199 |
| CF™532 | | | | 92180 | | | 92045 | | | 92104 | |
| CF™543 | | | | 92181 | | | 92044 | 92098 | | 92105 | 92172 |
| CF™555 | 92087 | 92038 | | 92081 | | 92153 | 92023 | | | 92130 | 96021 |
| 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 | | | | | | |
| CF™660C | 92095 | | | 92094 | | | 92028 | | 96001 | 92137 | |
| CF™660R | 96004 | 96010 | 92059 | 92182 | | 96024 | 92031 | | | 92134 | 92195 |
| CF™680 | 96005 | | | 92119 | | | 92029 | | 96003 | 92139 | |
| CF™680R | 96006 | | 92054 | | 92079 | 96025 | 92032 | | 96007 | 92107 | 92196 |
| CF™750 | | 92102 | | | | | | | | 92142 | |
| CF™770 | | 92065 | | | | 92192 | | | | 92150 | |
| CF™790 | | | | | | | | | | 92155* | |
| CF™800 | | | | | | | 92128 | | | 92127* | |

* For conjugation to aldehyde or ketone groups, we recommend using CF™ dye aminooxy forms.

* Unit size 0.25 umol

** Visit www.biotium.com to see our Tyramide Amplification Kits, containing CF™ dye tyramide and HRP secondary antibodies.

## 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 let us know. 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 and traditional reactive dyes, as well as sets of size- and charge-matched dyes.

# CF™ Dye Bioconjugates

## Bioconjugate applications

| Conjugate | Application |
|---|---|
| Annexin V | Phosphatidyl serine probe; apoptosis marker |
| α-Bungarotoxin | 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, anionic, fixable: MW 10K, 40K, 150K, 250K | Fluid-phase endocytosis tracer |
| Phalloidin | Filamentous actin probe |
| Peanut agglutinin (PNA) | Lectin; specific for terminal β-galactose |
| Streptavidin | Detection of biotinylated probes |
| Transferrin (human) | Recycling endosome tracer |
| Wheat germ agglutinin (WGA) | Lectin, binds N-acetyl-D-glucosamine and sialic acid; bacterial Gram stain, stains yeast bud scars |

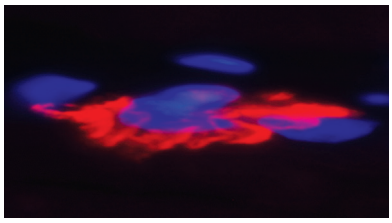

Figure 1. Frozen section of rat skeletal muscle stained with CF633 α-bungarotoxin (magenta) to detect nicotinic acetylcholine receptors at the neuromuscular junction. Nuclei are stained with DAPI (blue).

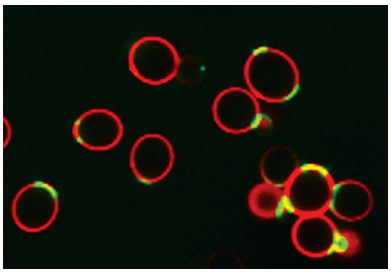

Figure 2. *S. cerevisiae* yeast stained with CF488A WGA and CF594 ConA. ConA (red) stains the cell wall, while WGA (green) preferentially stains bud scars.

## CF™ dye bioconjugates

| Conjugate | Annexin V | α-Bungarotoxin | BSA | Cholera Toxin B | Con A | Dextran 10,000 MW | Phalloidin | PNA | Streptavidin | Transferrin | WGA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CF™350 | 29012 | | | | 29015 | | 00049 | | 29031 | | 29021 |
| CF™405S | | 00002 | | | 29075 | | | | 29032 | | 29027 |
| CF™405M | 29009 | | | | 29074 | | 00034 | | 29033 | | 29028 |
| CF™405L | | | | | | | | | 29056 | | |
| CF™430 | | | | | | | 00054 | | 29065 | | |
| CF™440 | | | | | | | 00055 | | 29066 | | |
| CF™488A | 29005 | 00005 | 20289 | 00070 | 29016 | 80110 | 00042 | 29060 | 29034 | 00081 | 29022 |
| CF™532 | | | | 00074 | | | 00051 | | 29030 | | 29064 |
| CF™543 | | 00026 | | 00075 | | 80111 | 00043 | | 29043 | 00082 | |
| CF™555 | 29004 | 00018 | | | | 80112 | 00040 | | 29038 | | 29076 |
| CF™568 | 29010 | 00006 | | 00071 | | 80113 | 00044 | 29061 | 29035 | 00083 | 29077 |
| CF™594 | 29011 | 00007 | 20290 | 00072 | 29017 | 80114 | 00045 | 29062 | 29036 | 00084 | 29023 |
| CF™620R | | | | 00076 | | | | | | | |
| CF™633 | 29008 | 00009 | | 00077 | 29018 | | 00046 | | 29037 | | 29024 |
| CF™640R | 29014 | 00004 | 20291 | 00073 | 29019 | 80115 | 00050 | 29063 | 29041 | 00085 | 29026 |
| CF™647 | 29003 | | | | | | 00041 | | 29039 | | |
| CF™660C | | | | | | | 00052 | | | | |
| CF™660R | 29069 | | | 00078 | | | 00047 | | 29040 | | |
| CF™680 | 29007 | | 20292 | | 29020 | 80118 | 00053 | | | | 29029 |
| CF™680R | 29070 | 00003 | | 00079 | | 80116 | 00048 | | 29042 | 00086 | 29025 |
| CF™750 | 29006 | | | | | 80119 | | | | 00087 | |
| CF™770 | 29046 | | | | 29058 | 80120 | | | | | 29059 |
| CF™790 | 29047 | | | | | 80121 | | | | | |

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 for probe synthesis and TUNEL assay

| | CF™405S | 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 | 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

Our collection of monoclonal primary antibodies is constantly growing, visit www.biotium.com to see the most up-to-date offerings. Available in the following formats:

- Purified: 200 ug/mL in PBS with 0.05% BSA & 0.05% azide, 100 uL (20 ug) or 500 uL (100 ug) unit size
- Purified, BSA-free: 1 mg/ml in PBS with 0.05% azide, ready-to-use for Mix-n-Stain™ labeling (see p. 23) or other conjugation, 50 uL (50 ug) unit size
- Conjugates: Your choice of 12 CF™ dye colors for microscopy, flow cytometry, or near-infrared western blot, plus R-PE, APC, and PerCP; 100 ug/mL in PBS with 0.05% BSA & 0.05% azide, 100 uL (10 ug) or 500 uL (50 ug) unit size

## A
A, Forssman
ACTH
Adenosine Monophosphate Deaminase 3
AFP
Alkaline Phosphatase
AMACR / p504S
Androgen Receptor
Arginase 1

## B
Bax
BCL-10
bcl-2
bcl-6
bcl-x
Beta Catenin
Beta-2 Microglobulin
Biotin
Blood Group A
Blood Group Antigen A
Blood Group Antigen B
Bovine Serum Albumin
BrdU
Bromodeoxyuridine

## C
CA19-9
Caldesmon, HMW
Calgranulin B
Calponin-1
Calprotectin
Carbonic Anhydrase IX
Carcinoembryonic Antigen
CD10
CD100
CD104
CD106 / VCAM1
CD117
CD11a
CD11b
CD11c
CD13
CD14
CD146 / Mucin 18
CD147
CD15 / FUT4 / Lewis x
CD16
CD16 / Fc-gamma Receptor III
CD171 / L1CAM
CD176 / T-F Ag
CD18
CD19
CD195 / CCR-5
CD1a
CD1b
CD2
CD20
CD21
CD22
CD25
CD26
CD27
CD28
CD282 / TLR2
CD284 / TLR4
CD30
CD31 / PECAM-1
CD32
CD33
CD34
CD35 / CR1
CD36
CD37
CD38
CD3e
CD4
CD41a
CD43
CD44 Standard
CD45 / LCA
CD45RA
CD45RB
CD45RO
CD46
CD47
CD48
CD5
CD50
CD53
CD54
CD54 / ICAM-1
CD55
CD56 / NCAM
CD57 / B3GAT1
CD59
CD6
CD61
CD63
CD66
CD66, pan
CD68
CD7
CD70
CD71
CD74
CD79a
CD8
CD84
CD86
CD8A
CD8B
CD90
CD90 / Thy1
CD95
CD98
CD99
Cdc20
CDw17
CDw60
CDw75
CELA3B
Chromogranin A
CMV-p65
c-Myb
c-Myc
Complement C4d
Creatine Phosphokinase
Cyclin A2
Cyclin B1
Cyclin D1
Cytochrome C
Cytokeratin 10
Cytokeratin 10/13
Cytokeratin 14
Cytokeratin 17
Cytokeratin 18
Cytokeratin 19
Cytokeratin 5/8
Cytokeratin 6
Cytokeratin 7
Cytokeratin 8
Cytokeratin 8/18
Cytokeratin, Acidic
Cytokeratin, Basic
Cytokeratin, HMW
Cytokeratin, LMW
Cytokeratin, multi
Cytokeratin, pan

## D
DOG-1
Double Stranded DNA

## E
E-Cadherin / CD324
EGFR
EMI1
Eosinophil Peroxidase
Ep-CAM / CD326
Erythrocyte Specific
Estrogen Receptor
Estrogen Receptor beta 1

## F
Fascin-1
FGF23
Fibronectin
FOXP3
FSH beta

## G
GFAP
GITR / Tnfrsf18
GLG1
Glucose Regulated Protein 94
Glycophorin A / CD235a
Glypican-3
GM-CSF
GnRH-Receptor
Golgi Complex
gp100 / Melanosome
Granulocyte Marker
GCSF
Granzyme B

## H
HCG-alpha
HCG-beta
HCG-intact
Helicobacter pylori
Heparan Sulphate Proteoglycan
Hepatocyte Specific Antigen
HepPar-1
HER-2 / CD340
HIF1?
Histiocytoma Marker
Histone H1
HLA-Aw32 & HLA-A25
HLA-B
HLA-DRB
HSP27
HSP60
Human Nuclear Antigen
Human Nucleolar Antigen
Human Papillomavirus 16

## I
IDH1
IgA Immunoglobulin
IgA Secretory Component
IGF-1
IgG
IgG Immunoglobulin
IgM Immunoglobulin
IL-6
Insulin
Interferon alpha 1
Interferon alpha-2
Interferon gamma
Involucrin
IPO-38
Isotype control, mouse IgG1, κ
Isotype control, mouse IgG2a, κ
Isotype control, mouse IgG2b, κ

## K
Kappa Light Chain
Ksp-Cadherin / CDH16
Ku-Holo

## L
Lambda Light Chain
Laminin
LEC Chemokine
Lewis A
Lewis B
Liver Canuliculi
Lung Specific Antigen
Luteinizing Hormone beta

## M
Macrophage Specific Antigen
MAGE A1
Major Vault Protein
MALT-1
MAP3K1
MART-1 / Melan-A
MCAM / MUC18 / Mucin 18 / CD146
Melanoma Marker
MHC I
MHC II
Milk Fat Globulin
MiTF
Mitochondria
Mitochondrial Marker
Moesin
MRP-14
MUC18 / Mucin 18 / CD146
MUC2
MUC5AC
Mucin 1 / EMA / Episialin / CD227
Mucin 3
Mucin 5AC
Mucin 6
Muscle Specific Actin
Myeloid-Associated Differentiation Marker
MyoD1
Myogenin
Myosin, Smooth Muscle Heavy Chain

## N
Napsin-A
Neurofilament
Neurofilament, phospho
NGFR
NKX2.2
Nuclear Antigen
Nuclear Membrane
Nuclear Membrane Marker
Nucleolar / Nucleoli
Nucleolin
NuMA

## O
ODC-1

## P
p21 / WAF1
p24-HIV
p27 / KIP1
p34 / cdk1
p40
p53
p55;50 EBV-Early Antigen
p57 / KIP2
p57Kip2
PAX6
PAX7
PCNA
PD1 / PDCD1 / CD279
pgp9.5
Phosphotyrosine
PLAP
Plasma Cell Marker
PLGF
Pmel17 / gp100 / SILV
Podocalyxin
Progesterone
Progesterone Receptor
Prolactin Receptor
Prostate Specific Antigen
Proximal Nephrogenic Antigen
pS2
PSA
PTEN
PTH
PTH / Parathyroid Hormone

## R
Rabies
Retinol Binding Protein-1

## S
S100
S100A9
SHBG
Small Cell Lung Cancer
Smooth Muscle Actin
SOX10
SUMO-1
SUMO-2
SUMO-2/3

## T
TAG-72 / CA72.4
Tenascin
Testosterone
TGFalpha
TGF-beta
Thomsen-Friedenreich Ag.
Thymidylate Synthase
Thyroglobulin
TIMP3
TNF alpha
Topoisomerase I, MT
TOX3
TRAcP
Transgelin / SM22-alpha
Transglutaminase II
TRIM29
TRP1
TTF-1 / NKX2.1
Tyrosinase

## U
UACA / Nucling
UGT1A9
UPK3A

## V
VEGF-A
VEGFR1 / Flt-1
VEGFR2 / Flk-1
Vimentin

## W
von Willebrand Factor
WT1

## Z
ZAP70

# 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

| | Goat anti-GST | Mouse monoclonal anti-biotin | Mouse monoclonal anti-fluorescein | Mouse monoclonal anti-GFP | Mouse monoclonal anti-6X His tag | Rabbit anti-HA tag | Rabbit anti-RFP |
|---|---|---|---|---|---|---|---|
| Concentration/ Unit size | 1 mg/mL 0.1 mL | 2 mg/mL 50 uL or 0.25 mL | 2 mg/mL 50 uL or 0.25 mL | 1 mg/mL 0.1 mL | 1 mg/mL 0.1 mL | 1 mg/mL 50 uL | 1 mg/mL 0.1 mL |
| CF™405S | | 20203 | | | | | |
| CF™405M | | | 20214 | | | | |
| CF™488A | 20424 | 20204 | 20210 | 20215 | 20228 | 20238 | 20421 |
| CF™594 | 20425 | 20205 | 20211 | 20216 | 20229 | 20239 | 20422 |
| CF™633 | | 20206 | 20212 | 20217 | | | |
| CF™640R | 20426 | 20207 | 20213 | 20218 | 20237 | 20237 | 20423 |
| CF™647 | | | | | | | |
| CF™680 | | | | 20219 | 20359 | | |
| CF™770 | | 20367 | | 20220 | 20360 | | |

## Secondary antibodies, whole IgG (H+L), not highly 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)

| | Chicken anti-goat | Chicken anti-mouse | Chicken anti-rabbit | Goat anti-Guinea pig | 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 | | | | | 20080 | 20082 | | | | | | |
| CF™405M | | | | | 20180 | 20181 | | | | | | |
| CF™405L | | | | | 20408 | 20409 | | | | | | |
| CF™488A | 20225 | 20208 | 20209 | 20017 | 20010 | 20012 | 20028 | 20454 | 20449 | 20079 | 20021 | |
| CF™514 | | | | | 20386 | 20387 | | | | | | |
| CF™532 | | | | | 20365 | 20366 | | | | | | |
| CF™543 | 20333 | 20334 | 20335 | 20317 | 20306 | 20309 | 20324 | | | 20312 | 20315 | 20336 |
| CF™555 | | | | 20036 | 20030 | 20033 | 20236 | | | | 20031 | |
| CF™568 | 20337 | 20338 | 20339 | 20108 | 20100 | 20102 | 20091 | 20455 | 20450 | | 20107 | |
| CF™594 | 20226 | 20221 | 20223 | 20118 | 20110 | 20112 | 20160 | 20456 | 20451 | 20164 | 20117 | |
| CF™633 | 20227 | 20222 | 20224 | 20129 | 20120 | 20122 | 20138 | | | 20165 | 20128 | |
| CF™640R | | | | 20085 | 20197 | 20202 | 20089 | 20457 | 20452 | | | |
| CF™647 | | | | 20041 | 20040 | 20043 | 20286 | 20458 | 20453 | | | |
| CF™660C | | | | | 20050 | 20053 | | | | | | |
| CF™660R | | | | | 20054 | 20055 | | | | | | |
| CF™680 | | | | | | | | | | | 20068 | 20243 |
| CF™750 | | | | | 20070 | 20073 | | | | | | |
| CF™770 | | | | | | | | | | | | 20244 |
| CF™790 | | | | | 20378 | | | | | | | |

### 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 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 immunofluorence staining.

| | Donkey anti-mouse | Donkey anti-rabbit | Goat anti-mouse | Goat anti-rabbit |
|---|---|---|---|---|
| Min x react | Bv, Ch, Gt, GP, Hs, Hu, 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 |

## 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-CF™660R unit sizes: 0.5 mL, 50 uL, or 1 mg (lyophilized); CF™680-CF™790 available in 0.25 mL or 50 uL sizes

| | Bovine anti-goat | Donkey anti-chicken | Donkey anti-goat | Donkey anti-guinea pig | Donkey anti-human | Donkey anti-mouse | 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, 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 | | | | 20356 | | | 20420 | 20419 | |
| CF™405M | | | | 20376 | | | | | |
| CF™430 | | | | | | 20461 | 20462 | | |
| CF™488A | 20293 | 20166 | 20016 | 20169 | 20074 | 20014 | 20015 | 20027 | 20024 |
| 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 | | 20284 |
| CF™660C | | | 20051 | 20372 | | | | | |
| CF™660R | | | | | | 20388 | 20389 | 20390 | |
| CF™680 | | | 20060 | 20241 | 20278 | | 20418 | 20417 | 20062 |
| CF™680R | | | 20196 | | | 20194 | 20195 | | |
| CF™750 | | | 20362 | | | | 20298 | | |
| CF™770 | | | 20277 | 20242 | | | | | |
| CF™790 | | | 20345 | | 20279 | 20363 | 20344 | | |

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

See more highly cross-adsorbed secondaries on the next page

### 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 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-CF™660R unit sizes: 0.5 mL, 50 uL, or 1 mg (lyophilized); CF™680-CF™790 available in 0.25 mL or 50 uL sizes

| | Goat anti-chicken | 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 |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm | 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 | | | | | | | | | | |
| CF™405M | 20375 | | 20182 | 20340 | 20373 | 20374 | | | | |
| CF™430 | | | 20459 | | 20460 | | | | | |
| CF™488A | 20020 | 20022 | 20018 | 20302 | 20019 | 20023 | 20071 | 20026 | 20025 | 20172 |
| CF™543 | 20311 | 20319 | 20299 | 20328 | 20300 | 20321 | | 20307 | | 20323 |
| CF™555 | 20034 | 20320 | 20231 | | 20232 | 20233 | | 20235 | | |
| CF™568 | 20104 | 20097 | 20101 | 20301 | 20103 | 20096 | | 20093 | 20094 | |
| CF™594 | 20114 | 20154 | 20111 | 20303 | 20113 | 20155 | 20072 | 20158 | 20157 | 20173 |
| CF™633 | 20126 | 20132 | 20121 | 20341 | 20123 | 20133 | 20066 | 20136 | 20135 | 20174 |
| CF™640R | 20084 | 20081 | 20175 | 20304 | 20176 | 20088 | | 20200 | 20201 | |
| CF™647 | 20044 | 20280 | 20281 | | 20282 | 20283 | | 20285 | | |
| CF™660C | 20371 | | 20052 | 20356 | 20369 | 20370 | | | | |
| CF™660R | | | | | | | | | | |
| CF™680 | | 20287 | 20065 | | 20067 | 20069 | | 20061 | | |
| CF™680R | | | 20192 | | 20193 | | | | | |
| CF™750 | | | 20463 | | | | | | | |
| CF™770 | | 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

| | 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

Highly cross-adsorbed for multiple labeling (min X Bv, Hu, Rb)
2 mg/mL, unit size: 0.25 mL or 50 uL

| | Goat anti-mouse IgG1 | Goat anti-mouse IgG2a | Goat anti-mouse IgG2b |
|---|---|---|---|
| CF™350 | 20245 | 20255 | 20265 |
| CF™405S | 20380 | 20381 | 20382 |
| CF™488A | 20246 | 20256 | 20266 |
| CF™543 | 20325 | 20356 | 20327 |
| CF™555 | 20247 | 20257 | 20267 |
| 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 |
| CF™770 | 20254 | 20264 | 20274 |

## Goat anti-human isotype-specfic antibodies

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

| | 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 |
| CF™770 | | 20385 |

# Related Products and Accessories

*Buffers, mounting media, counterstains and more*

## RedDot™1 and RedDot™2 Far-Red Nuclear Stains

**RedDot™1**

- Replaces Draq®5 for live cell nuclear staining
- Cell normalization for In Cell Western®
- Cell cycle analysis by flow cytometry

**RedDot™2**

- Replaces Draq®7 for dead cell staining and fixed cell staining
- More specific than Draq®7 for fixed cell nuclear counterstaining

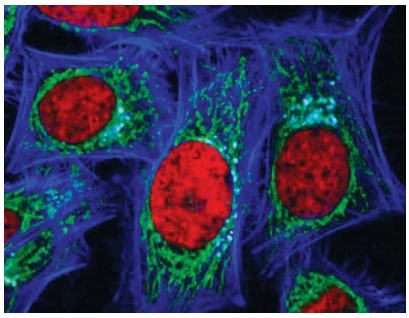

Figure 1. HeLa cells stained with rabbit anti-COX IV and CF488A goat anti-rabbit (mitochondria, green), and mouse anti-Golgin 97 and CF555 goat anti-mouse (Golgi, cyan) Actin filaments are stained with CF405 phalloidin (blue) and nuclei are stained with RedDot2 (red).

## EverBrite™ Mounting Media

- Superior antifade protection
- Compatible with CF™ dyes and other dyes
- Compatible with cyanine-based dyes (Cy® dyes, Alexa Fluor® 647 and DyLight® 649), unlike VectaShield®
- Available in wet-set or hardset formulations
- With or without DAPI

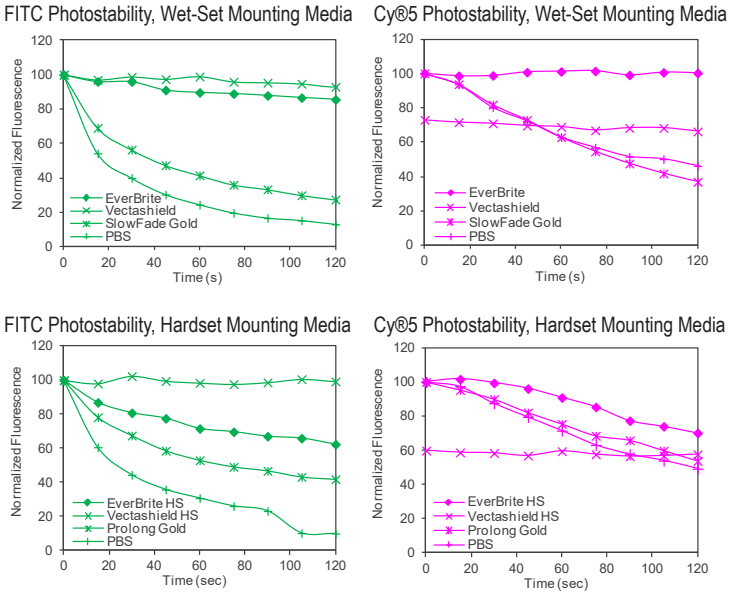


Figure 2. Photostability of HeLa cell immunofluorescence staining in various mounting media. A major advantage of EverBrite™ mounting medium is that, unlike Vectashield®, it does not decrease the fluorescence of cyanine-based fluorophores. Fluorescence values for Cy®5 in Vectashield® media are normalized to PBS time 0 to illustrate the drop in fluorescence of cyanine dyes caused by Vectashield®.

## CoverGrip™ Coverslip Sealant

- Designed specifically for sealing coverslip edges
- Won't mix with aqueous mounting medium like nail polish can
- Made with natural Limonene solvent
- 15 mL brush bottle, or 100 mL refill

Figure 3. CoverGrip Coverslip Sealant brush bottle and refill bottle.

## Super$^{HT}$ Pap Pens

- Create hydrophobic barriers on glass slides to separate specimens and conserve antibodies
- Insoluble in aqueous buffers, detergents, alcohol, or acetone, removable with xylene
- Stable at temperatures up to 120°C
- Super$^{HT}$ Mini Pap Pen: 2.5 mm tip, ~400 applications
- Super$^{HT}$ Pap Pen: 4 mM tip, ~800 applications

Figure 4. Super$^{HT}$ Pap Pens.

# Related Products and Accessories

## TrueBlack™ Lipofuscin Autofluorescence Quencher

- Eliminates autofluorescence from lipofuscin in human and aged animal tissue sections
- Reduces autofluorescence from other sources, such as red blood cells and collagen/elastin
- Less red/far-red background compared to Sudan Black B
- Can be used to treat tissue sections before or after antibody staining

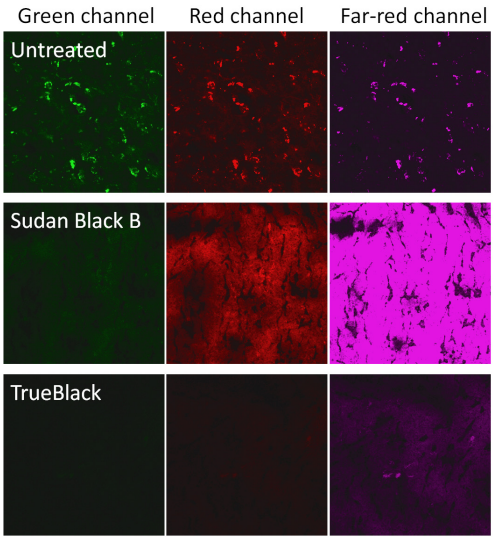


Figure 3. Lipofuscin autofluorescence in human cerebral cortex sections. Top row: lipofuscin appears as highly fluorescent granules throughout the tissue section in all three fluorescent channels. Middle row: Sudan Black B masks lipofuscin autofluorescence, but causes high background in the red and far-red channels. Bottom row: TrueBlack quenches lipofuscin with minimal increase in fluorescence background (bottom row).

## Accessory Products

| Catalog No. | Product Name |
|---|---|
| 23001 | EverBrite™ Mounting Medium |
| 23002 | EverBrite™ Mounting Medium with DAPI |
| 23003 | EverBrite™ Hardset Mounting Medium |
| 23004 | EverBrite™ Hardset Mounting Medium with DAPI |
| 23005 | CoverGrip™ Coverslip Sealant |
| 23007 | TrueBlack™ Lipofuscin Autofluorescence Quencher, 20X in DMF |
| 40060 | RedDot™1 Far Red Nuclear Counterstain, 200X in $H_2O$ |
| 40061 | RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO |
| 30069 | AccuEasy™ Flow Cytometry Kit |
| 22003 | Mini Cell Scrapers |
| 22005 | Mini Super$^{HT}$ Pap Pen |
| 22006 | Super$^{HT}$ Pap Pen |
| 23006 | Flow Cytometry Fixation/Permeabilization Kit |
| 22015 | Fixation Buffer |
| 22016 | Permeabilization Buffer |
| 22017 | Permeabilization and Blocking Buffer (5X) |
| 22010 | 10X Fish Gelatin Blocking Agent |

## AccuEasy™ Flow Cytometry Kit

- Stain and harvest adherent cells for flow cytometry
- Prevents loss of surface marker staining upon cell detachment
- Increases sensitivity of cell surface marker detection compared to conventional methods

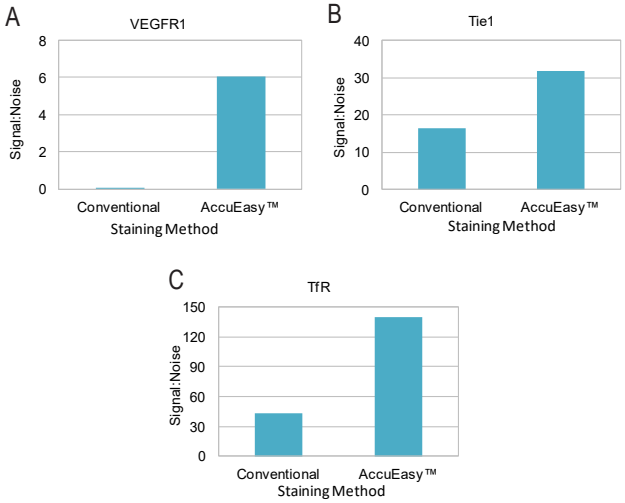


Figure 1. The AccuEasy staining method improves signal to noise ratio in flow cytometry analysis of cell surface markers on adherent cells compared conventional methods. A. Indirect immunofluorescence staining of EA.hy926 human endothelial cells with mouse monoclonal antibody against VEGF Receptor 1 (VEGFR1) or isotype control and PE conjugated anti-mouse IgG. B. Staining of EA.hy926 cells with mouse monoclonal antibody against Tie1 or isotype antibody control and PE conjugated anti-mouse IgG. C. Direct immunofluorescence staining of HeLa cells with Mix-n-Stain™ CF™488A labeled mouse monoclonal antibody against transferrin receptor (TfR), or isotype control.

## Mini Cell Scrapers

- For harvesting cells from 96-, 48-, or 24-well plates
- 0.5 cm wide and 6 cm long polystyrene scrapers
- Sterile and disposable

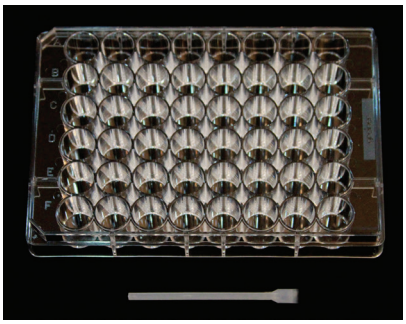

Figure 2. Mini Cell Scraper shown above next to a 48-well plate for scale.

**Visit www.biotium.com to find more buffers, counterstains, and accessories.**

**Biotium, Inc.**

Toll Free: 800-304-5357
Phone: 510-265-1027
Fax: 510-265-1352

General Inquiries
btinfo@biotium.com

Quotes and Ordering
order@biotium.com

Technical Support
techsupport@biotium.com

Biotium