



# Near-IR Dyes

## For NIR western and *in vivo* imaging

**Next-generation NIR dyes with superior water-solubility, brightness, and photostability**

### Industry Leading Near-IR Dyes

- Brighter than near-IR Alexa Fluor®, Cy®, and IR® Dyes
- Unrivaled photostability
- Highly water soluble without excessive negative charge
- Insensitive to pH
- Excellent labeling efficiency with amine-reactive SE forms
- Largest selection of wavelengths
- Multiple dyes validated for STORM or STED super-resolution imaging
- Compatible with popular instruments like IVIS®, FMT®, LI-COR Odyssey®, Olympus OV-100, Andor Dragonfly, and others

Near-infrared (also called near-IR or NIR) dyes offer important advantages over traditional visible light dyes. Near-IR detection can be highly sensitive and specific, because biological samples have minimal autofluorescence in near-IR wavelengths. In addition, near-IR fluorescence emission has strong tissue penetration ideal for small animal *in vivo* imaging. Near-IR dyes are also used for highly sensitive multiplex western and In-Cell Western™ assays.

Near IR dyes are typically large aromatic organic molecules with poor water solubility. To improve the solubility for biological applications, chemists traditionally attach a high number of negatively charged sulfonate groups to the dyes. While this dye design approach overcomes the solubility problem, it also introduce a major problem; when these highly negatively charged dyes are attached to antibodies, the isoelectric points of the antibodies can be dramatically changed, resulting in high non-specific binding (see Fig. 5 inside). Moreover, such highly charged dyes or their protein conjugates tend to be more immunogenic for *in vivo* applications.

Biotium's scientists invented a unique solution to this problem. Unlike the near-IR dyes from our competitors, Biotium's near-IR CF® dyes are modified with highly water soluble yet neutral polyethylene glycol (PEG) groups to replace some of the sulfonate groups, rendering the dyes highly water soluble (Fig. 4). The PEG groups not only minimize the number of sulfonate groups necessary but also help shield the remaining charges on the dyes from being fully exposed. Consequently, a higher degree of labeling (number of dyes per protein) can be achieved for brighter conjugates (Figs. 2-3) without sacrificing antibody specificity (Fig. 5). Moreover, pegylated near IR CF® dyes are also brighter, less immunogenic, and more stable *in vivo* than other commercial near IR dyes (Fig. 10).

In addition, amine-reactive near-IR CF® dyes succinimidyl esters have much higher labeling efficiency than other near-IR dyes because of their excellent solubility and high reactivity (generally >95%).

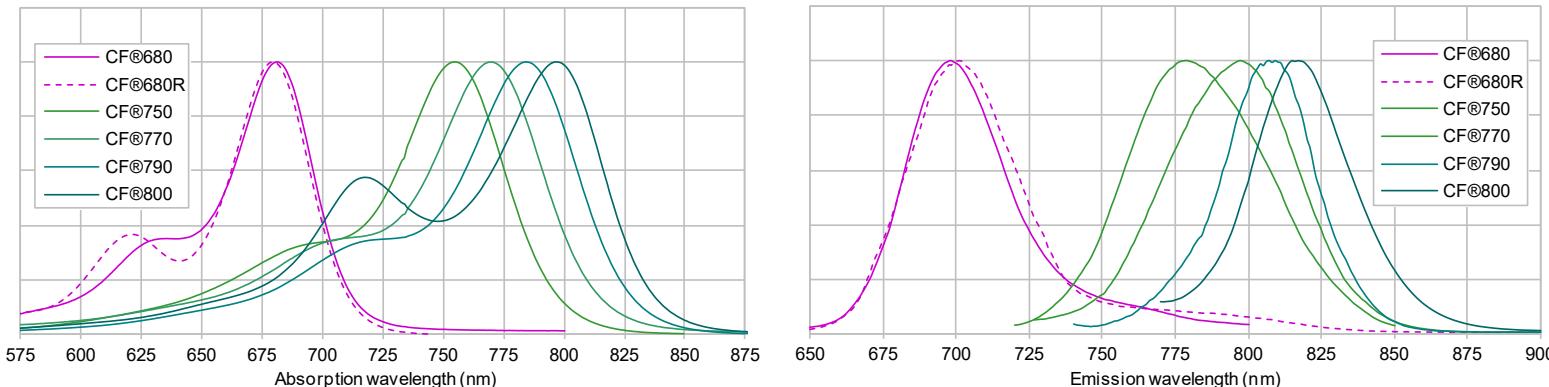


Figure 1. Absorption and emission spectra of near-IR CF® dyes.

# Near-IR CF® Dye Advantages

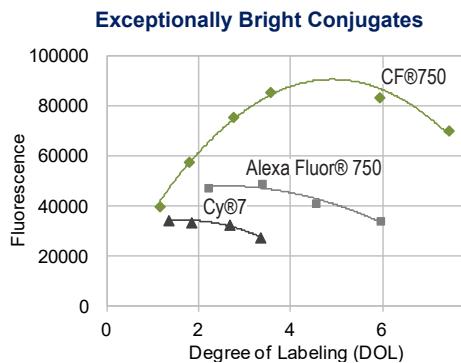


Figure 2. Relative fluorescence of goat anti-mouse IgG conjugates labeled with CF®750, Alexa Fluor® 750 or Cy®7. Because CF®750 is highly water soluble, a higher degree of labeling (number of dyes per protein) can be achieved without fluorescence quenching, resulting in brighter conjugates.

## Higher Signal for NIR Western on LI-COR® Odyssey®

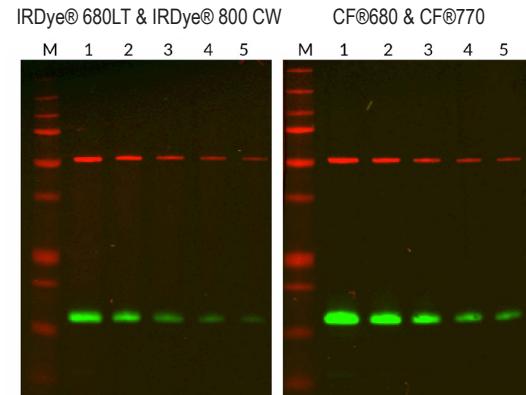


Figure 3. CF® dye conjugates are brighter than IRDye® conjugates for NIR western. Western blotting of HeLa cell lysate (2  $\mu$ g to 0.125  $\mu$ g, lanes 1-5) for tubulin and COX IV, detected by IR®680LT or CF®680 (red) and IRDye® 800CW or CF®770 (green) secondary antibodies. Quantitation of bands showed approximately 50% brighter signal with CF® dyes compared to IRDye®. M: molecular weight marker.

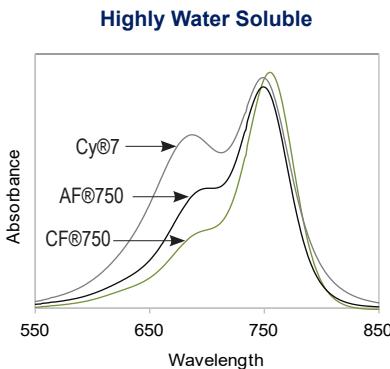


Figure 4. Normalized absorbance spectra of goat anti-mouse IgG labeled with CF®750, Alexa Fluor® 750 (AF®750) or Cy®7. Cy®7 and Alexa Fluor® 750 have large shoulder peaks (arrows), which are indicative of dye aggregation due to poor solubility. Dye aggregates tend to self-quench and therefore don't contribute to fluorescence.

## Superior Conjugate Specificity

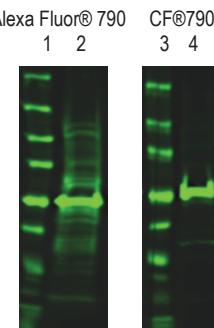


Figure 5. Near-IR CF® dyes carry less negative charge than other near-IR dyes, resulting in less non-specific binding of conjugates. Western blot detection of tubulin in HeLa cell lysate using secondary antibodies conjugated to Alexa Fluor® 790 (AF790) (lane 2) or CF®790 (lane 4). Lanes 1 and 3 contain molecular weight marker.

## Remarkably Photostable

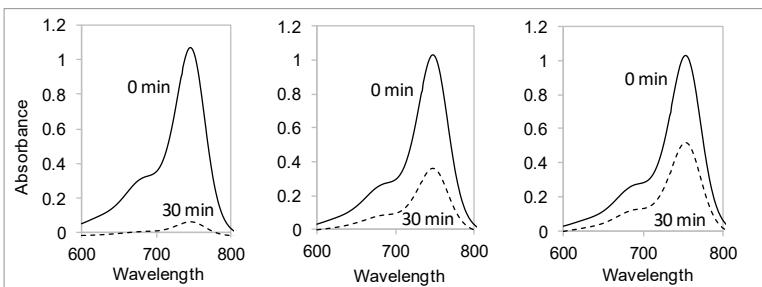


Figure 6. Photostability of Cy®7, Alexa Fluor® 750 and CF®750 dyes. Plots show absorption spectra of the respective dyes before (solid line) and after (dashed line) 30 minutes of sunlight exposure.

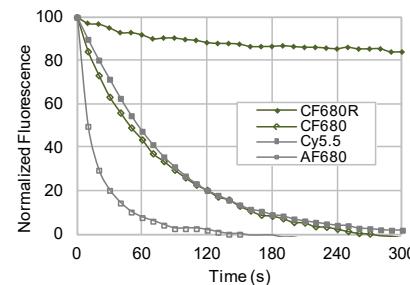


Figure 7. CF®680R is highly photostable. Immunofluorescence was performed for CD3 in Jurkat cells using secondary antibody conjugates of the indicated dyes. Cells were exposed to continuous mercury arc lamp excitation with a Cy®5 filter set. Images were captured every 10 seconds for 5 minutes; mean fluorescence was normalized to time 0.

# Near-IR CF® Dye Applications

## Super Resolution Microscopy

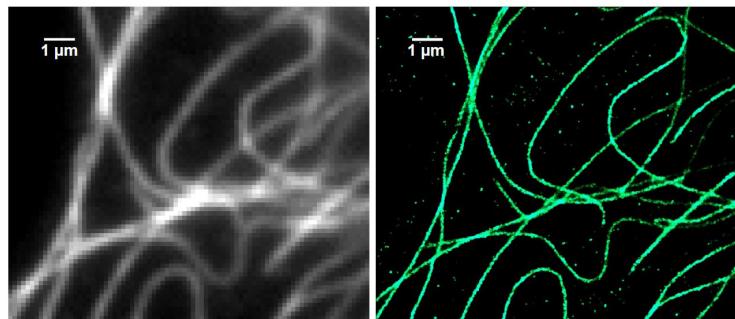


Figure 8. Comparison of microtubule imaging using conventional wide-field microscopy (left) with STORM (right) using CF®680 dye conjugate. Images courtesy of Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

## Near-IR CF® Dye Product Lines

- Primary antibodies, secondary antibodies, and other bioconjugates
- Annexin V conjugates (preservative-free)
- VivoBrite™ Rapid Antibody Labeling Kits for Small Animal Imaging
- Amine-reactive succinimidyl esters and protein labeling kits
- Mix-n-Stain™ Antibody Labeling Kits

## In-Cell Western™ on LI-COR® Odyssey®

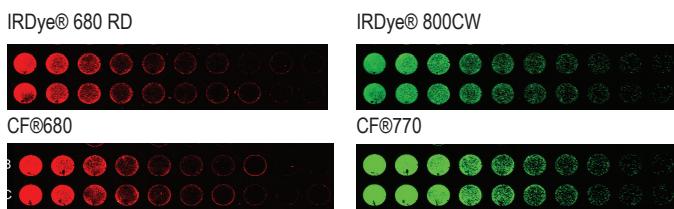


Figure 9. Comparison of CF® Dye and IRDye® secondary antibody conjugates by In Cell Western™ using the LI-COR Odyssey near-IR imaging system. Two-fold dilutions of HeLa cells were grown in 96-well tissue culture plates in duplicate wells. Intracellular immunofluorescence was performed for tubulin and COXIV with either CF® dyes or IRDye® conjugated secondary antibodies. The plate was scanned using a LI-COR® Odyssey® near-infrared imaging system. Fluorescence quantitation showed that the CF® dye conjugates staining had two-fold higher signal compared to the respective IRDye® conjugates.

## In Vivo Imaging

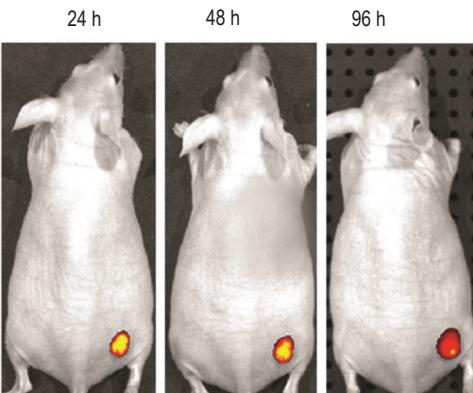


Figure 10. Tumors in mice were imaged using an IVIS® imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin® conjugated to CF®750. Images courtesy of Caliper Life Sciences.

Table 1. Near-Infrared CF® Dyes

Dye	Ex/Em	Replacement for	Features
CF®680	681/698 nm	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	<ul style="list-style-type: none"><li>CF®680 is the brightest among spectrally similar dyes</li><li>Recommended for protein or antibody labeling</li><li>Matches the 700 channel of LI-COR® Odyssey®</li><li>Validated in multicolor 3D STORM imaging with CF®568, CF®647, and CF®660C</li></ul>
CF®680R	680/701 nm	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	<ul style="list-style-type: none"><li>CF680R is the most photostable 680 nm dye</li><li>Molecular weight suitable for labeling small molecules like nucleic acids</li><li>Matches the 700 channel of LI-COR® Odyssey®</li><li>Validated in STED, single-molecule spectroscopy, and multi-color STORM imaging</li></ul>
CF®750	755/777 nm	Alexa Fluor® 750, Cy®7, DyLight® 750, IRDye® 750	
CF®770	770/797 nm	DyLight® 800, IRDye® 800CW	
CF®790	784/806 nm	Alexa Fluor® 790	<ul style="list-style-type: none"><li>Exceptionally bright and stable</li><li>Highly water soluble without bearing excessive charge</li><li>CF®750 is validated in super-resolution imaging by STORM</li><li>Compatible with LI-COR® Odyssey®; CF®770 matches the 800 channel</li></ul>
CF®800	797/816 nm	Spectrally similar to Indocyanine Green	

# Near-IR Dye Products

## Near-IR CF® Dye Reactive Dyes and Protein Labeling Kits

Succinimidyl esters can be used to covalently primary amines, such as lysine residues in proteins. CF® Dye Protein Labeling Kits include everything you need to label and purify 3 reactions of 1 mg protein each. VivoBrite® Rapid Antibody Labeling Kits for Small Animal Imaging also include filter sterilization to prepare conjugates for *in vivo* imaging.

Product	Ex/Em	Unit Size	Cat. #
CF®680 Succinimidyl Ester	681/698 nm	1 umol	92139
CF®680R Succinimidyl Ester	680/701 nm	1 umol	92107
CF®750 Succinimidyl Ester	755/777 nm	1 umol	92142
CF®770 Succinimidyl Ester	770/797 nm	1 umol	92150
CF®790 Succinimidyl Ester	784/806 nm	0.25 umol	92155
CF®800 Succinimidyl Ester	797/816 nm	0.25 umol	92127
CF®680 SE Protein Labeling Kit	681/698 nm	3 labelings	92220
CF®680R SE Protein Labeling Kit	680/701 nm	3 labelings	92226
CF®750 SE Protein Labeling Kit	755/777 nm	3 labelings	92221
CF®770 SE Protein Labeling Kit	770/797 nm	3 labelings	92222
VivoBrite™ CF®680 Antibody Labeling Kit	681/698 nm	3 labelings	92160
VivoBrite™ CF®750 Antibody Labeling Kit	755/777 nm	3 labelings	92161
VivoBrite™ CF®770 Antibody Labeling Kit	770/797 nm	3 labelings	92162
VivoBrite™ CF®790 Antibody Labeling Kit	784/806 nm	3 labelings	92163

## Near-IR CF® Dye Mix-n-Stain™ Antibody Labeling Kits

Mix-n-Stain™ Antibody Labeling Kits allow you to label between 5 and 100  $\mu$ g of antibody in just 30 minutes with no purification step. The labeling is covalent and stable, and the reaction tolerates common antibody storage buffer components.

Product	Dye	5-20 $\mu$ g labeling	20-50 $\mu$ g labeling	50-100 $\mu$ g labeling
CF® Dye Mix-n-Stain™ Antibody Labeling Kits	CF®680	92282	92262	92240
	CF®680R	92283	92263	92246
	CF®750	92284	92264	92241
	CF®770	92285	92265	92242
	CF®790	92288	92268	92248

## Primary and Secondary Antibody Conjugates

Visit [biotium.com](http://biotium.com) to browse or search our growing collection of more than 1000 monoclonal primary antibodies with 19 conjugation options, including our near-IR CF®680, CF®680R, and CF®770. We also supply a wide selection of anti-tag antibodies and secondary antibodies conjugated to our near-IR CF® dyes. Single-label secondary antibodies optimized for STORM imaging are available with our STORM-compatible CF®680 and CF®750 dyes.

## Other Bioconjugates

A variety of bioconjugates and lectins are available conjugated to our near-IR CF® dyes for cell labeling and tracing, including dextrans, streptavidin, transferrin, WGA, and others. Visit [biotium.com](http://biotium.com) to learn more.

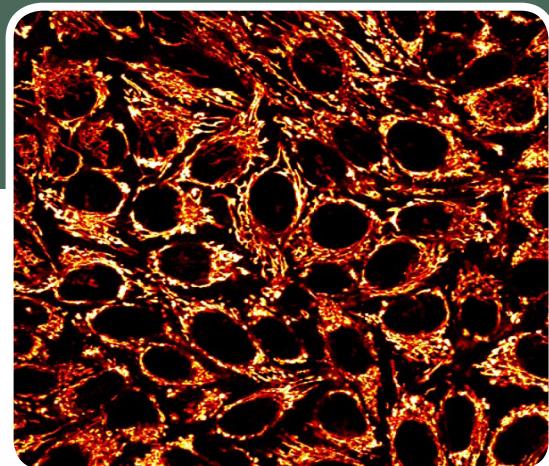


Figure 11. Live cell mitochondrial staining with MitoView™ 720.

## Near-IR CF® Dye Annexin V Conjugates

Fluorescent Annexin V binds phosphatidylserine on the surface of apoptotic cells. Near-IR CF® dye Annexin V conjugates are preservative-free lyophilized solids compatible with *in vivo* use.

Product	Ex/Em	Unit Size	Cat. #
CF®680 Annexin V	681/698 nm	25 $\mu$ g	29007
CF®750 Annexin V	755/777 nm	25 $\mu$ g	29006
CF®770 Annexin V	770/797 nm	25 $\mu$ g	29046
CF®790 Annexin V	784/806 nm	25 $\mu$ g	29047
CF®800 Annexin V	797/816 nm	25 $\mu$ g	29078

## Near-IR Cytoplasmic Membrane Dyes

CellBrite™ NIR Cytoplasmic Membrane Dyes are ready-to-use dye delivery solutions that can be added directly to normal culture media to uniformly label cells in suspension or adherent cultures. They are near-IR lipophilic carbocyanine dyes, which have low cytotoxicity and high resistance to intercellular transfer. The dyes are optimally detected by near-IR imaging, but are also bright enough to be detected in the Cy®5 channel. DiR is a classic near-infrared carbocyanine dye used for *in vivo* imaging.

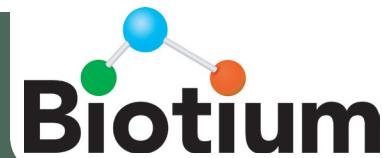
Product	Ex/Em	Unit Size	Cat. #
CellBrite™ NIR680 Cytoplasmic Membrane Dye	683/724 nm	100 $\mu$ L	30070
CellBrite™ NIR750 Cytoplasmic Membrane Dye	748/780 nm	100 $\mu$ L	30077
CellBrite™ NIR770 Cytoplasmic Membrane Dye	767/806 nm	100 $\mu$ L	30078
CellBrite™ NIR790 Cytoplasmic Membrane Dye	786/820 nm	100 $\mu$ L	30079
DiR (DiIC18(7))	748/780 nm	25 mg	60017

## MitoView™ 720 Mitochondrial Dye

MitoView™ 720 is a unique fluorogenic mitochondrial stain for live cells. The dye is optimally detected by near-IR imaging, but due to its brightness it also can be detected in the Cy®5 channel.

Product	Ex/Em	Unit Size	Cat. #
MitoView™ 720	720/798 nm	50 $\mu$ g	70068-T
		20 x 50 $\mu$ g	70068

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