

# Near-IR Dyes

## For NIR Western & *In Vivo* Imaging

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

#### Industry Leading Near-IR Dyes

- Brighter compared to other commercially available near-IR dyes
- 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<sup>®</sup>, FMT<sup>®</sup>, LI-COR Odyssey<sup>®</sup>, 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<sup>™</sup> 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 approach helps to address issues with solubility, it also introduces a major problem. When these highly negatively charged dyes are attached to antibodies, the isoelectric points of the antibodies are dramatically altered, resulting in high non-specific binding (see Fig. 5 inside). Moreover, 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 other commercially available near-IR dyes, Biotium's near-IR CF<sup>®</sup> Dyes are modified with highly water-soluble yet neutral polyethylene glycol (PEG) groups to replace certain 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<sup>®</sup> 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<sup>®</sup> Dye succinimidyl esters have much higher labeling efficiency than other near-IR dyes because of their excellent solubility and high reactivity (generally >95%).

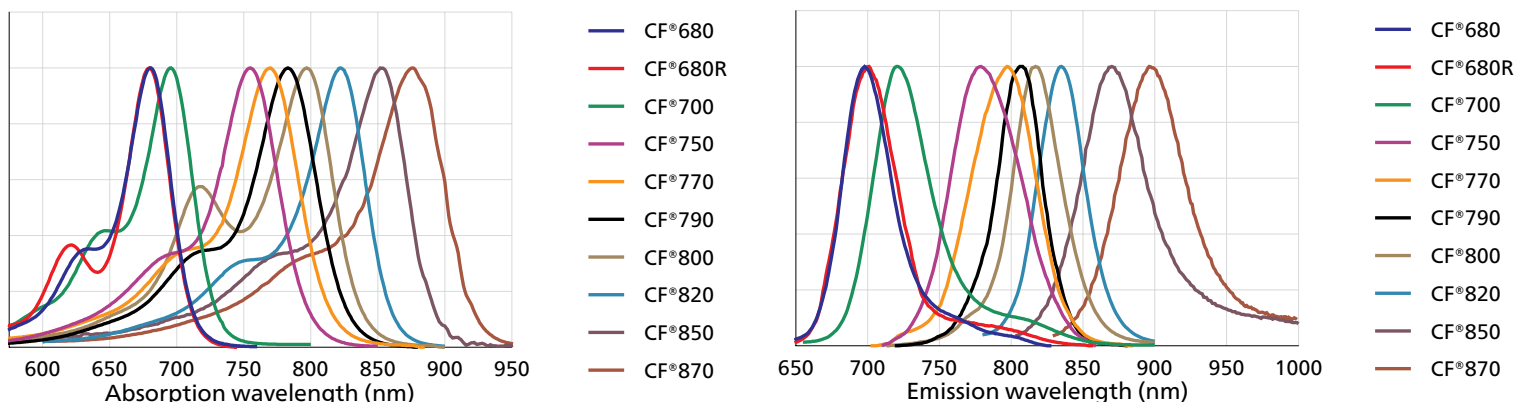


Figure 1. Absorption and emission spectra of near-IR CF<sup>®</sup> Dyes.

# Near-IR CF<sup>®</sup> Dye Advantages

## Exceptionally Bright Conjugates

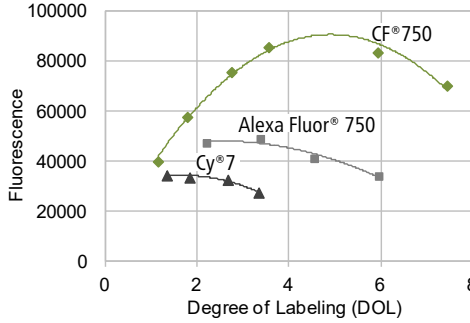


Figure 2. Relative fluorescence of goat anti-mouse IgG conjugates labeled with CF<sup>®</sup>750, Alexa Fluor<sup>®</sup> 750 or Cy<sup>®</sup>7. Because CF<sup>®</sup>750 is highly water-soluble, a higher degree of labeling (number of dyes per protein) can be achieved without fluorescence quenching,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

## Highly Water Soluble

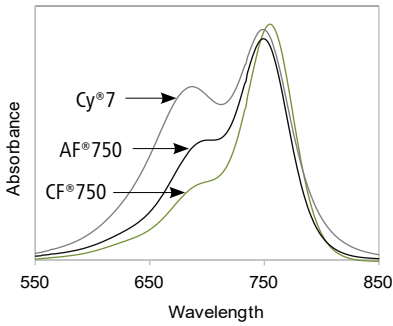


Figure 4. Normalized absorbance spectra of goat anti-mouse IgG labeled with CF<sup>®</sup>750, Alexa Fluor<sup>®</sup> 750 (AF<sup>®</sup>750) or Cy<sup>®</sup>7. Cy<sup>®</sup>7 and Alexa Fluor<sup>®</sup> 750 have large shoulder peaks (arrows), which are indicative of dye aggregation due to poor solubility.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

## Remarkably Photostable

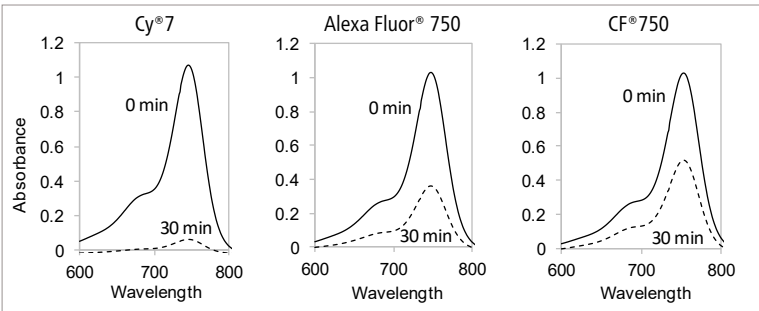


Figure 6. Photostability of Cy<sup>®</sup>7, Alexa Fluor<sup>®</sup> 750 and CF<sup>®</sup>750 dyes. Plots show absorption spectra of the respective dyes before (solid line) and after (dashed line) 30 minutes of sunlight exposure.

## Higher Signal for NIR Western on LI-COR<sup>®</sup> Odyssey<sup>®</sup>

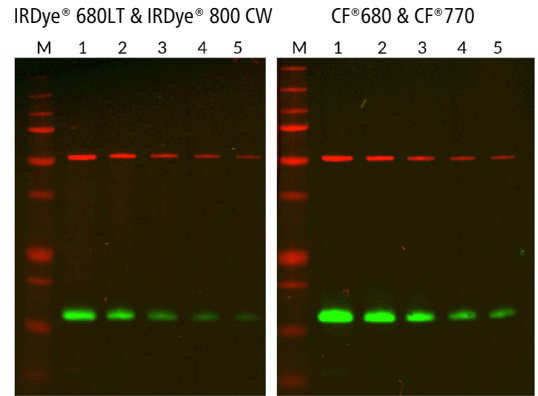


Figure 3. CF<sup>®</sup> Dye conjugates are brighter than IRDye<sup>®</sup> conjugates for NIR western. Western blotting of HeLa cell lysate (2 ug to 0.125 ug, lanes 1-5) for tubulin and COX IV, detected by IRDye<sup>®</sup>680LT or CF<sup>®</sup>680 (red) and IRDye<sup>®</sup> 800CW or CF<sup>®</sup>770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

## Superior Conjugate Specificity

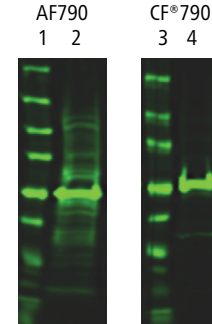


Figure 5. Near-IR CF<sup>®</sup> Dyes carry less negative charge than other near-IR dyes, resulting in less non-specific binding of conjugates. Western blot detection of tubulin in HeLa cell lysate using secondary antibodies conjugated to Alexa Fluor<sup>®</sup> 790 (AF790) (lane 2) or CF<sup>®</sup>790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

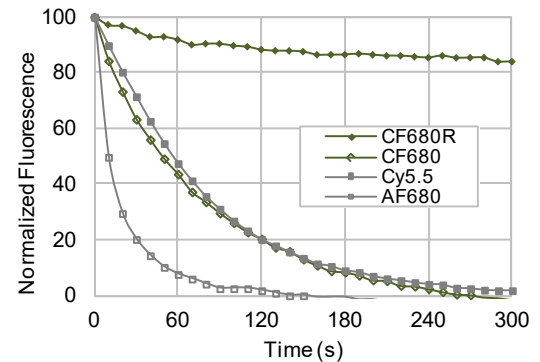


Figure 7. CF<sup>®</sup>680R is highly photostable. Immunofluorescence was performed for CD3 in Jurkat cells using secondary antibody conjugates of the indicated dyes. Cells were exposed to continuous mercury arc lamp excitation with a Cy<sup>®</sup>5 filter set. Images were captured every 10 seconds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

# Near-IR CF<sup>®</sup> Dye Applications

## Super Resolution Microscopy

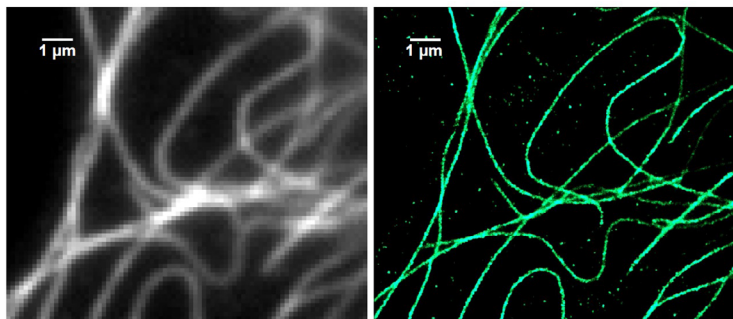


Figure 8. Comparison of microtubule imaging using conventional wide-field microscopy (left) with STORM (right) using CF<sup>®</sup>680 dye conjugate. Images courtesy of Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

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

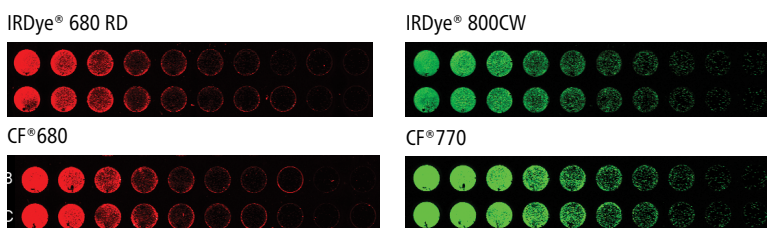


Figure 9. Comparison of CF<sup>®</sup> Dye and IRDye<sup>®</sup> secondary antibody conjugates by In Cell Western™ using the LI-COR Odyssey near-IR imaging system. Two-fold dilutions of HeLa cells were grown in 96-well tissue culture plates in duplicate wells. Intracellular immunofluorescence was performed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

## Table 1. Near-Infrared CF<sup>®</sup> Dyes

Dye	Ex/Em (nm)	Replacement for	Features
CF <sup>®</sup> 680	681/698	Alexa Fluor <sup>®</sup> 680, Cy <sup>®</sup> 5.5, DyLight <sup>®</sup> 680, IRDye <sup>®</sup> 680LT	<ul style="list-style-type: none"> <li>CF<sup>®</sup>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<sup>®</sup> Odyssey<sup>®</sup></li> <li>Validated in multicolor 3D STORM imaging with CF<sup>®</sup>568, CF<sup>®</sup>647, and CF<sup>®</sup>660C</li> </ul>
CF <sup>®</sup> 680R	680/701	Alexa Fluor <sup>®</sup> 680, Cy <sup>®</sup> 5.5, DyLight <sup>®</sup> 680, IRDye <sup>®</sup> 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<sup>®</sup> Odyssey<sup>®</sup></li> <li>Validated in STED, single-molecule spectroscopy, and multi-color STORM imaging</li> </ul>
CF <sup>®</sup> 750	755/777	Alexa Fluor <sup>®</sup> 750, Cy <sup>®</sup> 7, DyLight <sup>®</sup> 750, IRDye <sup>®</sup> 750	<ul style="list-style-type: none"> <li>Exceptionally bright and stable</li> <li>Highly water soluble without bearing excessive charge</li> <li>CF<sup>®</sup>750 is validated in super-resolution imaging by STORM</li> <li>Compatible with LI-COR<sup>®</sup> Odyssey<sup>®</sup>; CF<sup>®</sup>770 matches the 800 channel</li> </ul>
CF <sup>®</sup> 770	770/797	DyLight <sup>®</sup> 800, IRDye <sup>®</sup> 800CW	
CF <sup>®</sup> 790	784/806	Alexa Fluor <sup>®</sup> 790	
CF <sup>®</sup> 800	797/816	Spectrally similar to Indocyanine Green	
CF <sup>®</sup> 820	822/835	DY-820	
CF <sup>®</sup> 850	852/870	Unique dye	<ul style="list-style-type: none"> <li>CF<sup>®</sup>850 and CF<sup>®</sup>870 are unique near-IR dyes designed for the 808 nm laser</li> </ul>
CF <sup>®</sup> 870	876/896	Unique dye	

CF Dye technology is covered by US and international patents. Alexa Fluor and DyLight are registered trademarks of Thermo Fisher Scientific. Avastin is a registered trademark of Genentech, Inc. Cy dye is a registered trademark of Cytiva. IRDye and Odyssey are registered trademarks and In-Cell Western is a trademark of LI-COR, Inc. IVIS and FMT are registered trademarks of PerkinElmer Inc.

## Near-IR Dyes

## Near-IR CF<sup>®</sup> Dye Product Lines

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

## In Vivo Imaging

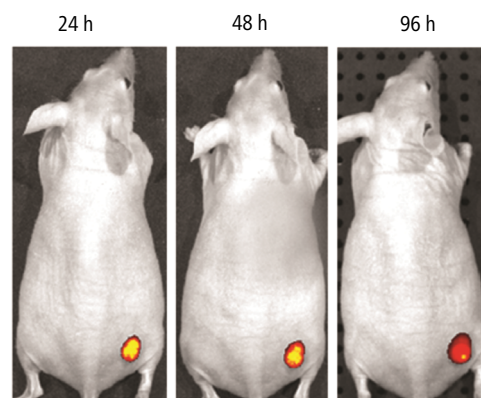


Figure 10. Tumors in mice were imaged using an IVIS<sup>®</sup> imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin<sup>®</sup> conjugated to CF<sup>®</sup>750. Images courtesy of Caliper Life Sciences.

# Near-IR Dye Products

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

Product	Ex/Em (nm)	Unit Size	Cat. No.
CF®680 Succinimidyl Ester	681/698	1 umol	92139
CF®680R Succinimidyl Ester	680/701	1 umol	92107
CF®750 Succinimidyl Ester	755/777	1 umol	92142
CF®770 Succinimidyl Ester	770/797	1 umol	92150
CF®790 Succinimidyl Ester	784/806	0.25 umol	92155
CF®800 Succinimidyl Ester	797/816	0.25 umol	92127
CF®820 Succinimidyl Ester	822/835	0.25 umol	96068
CF®850 TFP	852/870	0.25 umol	96094
CF®870 TFP	876/896	0.25 umol	96095
CF®680 SE Protein Labeling Kit	681/698	3 labelings	92220
CF®680R SE Protein Labeling Kit	680/701	3 labelings	92226
CF®750 SE Protein Labeling Kit	755/777	3 labelings	92221
CF®770 SE Protein Labeling Kit	770/797	3 labelings	92222
VivoBrite™ CF®680 Antibody Labeling Kit	681/698	3 labelings	92160
VivoBrite™ CF®750 Antibody Labeling Kit	755/777	3 labelings	92161
VivoBrite™ CF®770 Antibody Labeling Kit	770/797	3 labelings	92162
VivoBrite™ CF®790 Antibody Labeling Kit	784/806	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 ug of antibody in just 15 minutes with no purification step. The labeling is covalent and stable, and the reaction tolerates common antibody storage buffer components.

Product	Dye	5-20 ug labeling	20-50 ug labeling	50-100 ug 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
	CF®800	92428	92429	92430
	CF®820	92431	92432	92433

## Secondary Antibody Conjugates

Visit [biotium.com](http://biotium.com) to browse our 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.

## NucSpot® Nuclear Stains

Nuclear-specific counterstains ideal for fixed cells or staining dead cells in live cultures with enhanced photostability compared to commonly used alternatives. Available in 2 near-IR colors for Cy®5.5 and Cy®7 channels.

Product	Ex/Em (nm)	Unit Size	Cat. No.
NucSpot® 680/700, 1000X in DMSO	683/707	20 uL	41035-T
		100 uL	41035
NucSpot® 750/780, 1000X in DMSO	757/780	20 uL	41038-T
		100 uL	41038

## 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 (nm)	Unit Size	Cat. No.
CF®680 Annexin V	681/698	25 ug	29007
CF®680R Annexin V	699/737	25 ug	29070
CF®700 Annexin V	699/737	25 ug	29082
CF®750 Annexin V	755/777	25 ug	29006
CF®770 Annexin V	770/797	25 ug	29046
CF®790 Annexin V	784/806	25 ug	29047
CF®800 Annexin V	797/816	25 ug	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. No.
CellBrite™ NIR680 Cytoplasmic Membrane Dye	683/724 nm	100 uL	30070
CellBrite™ NIR750 Cytoplasmic Membrane Dye	748/780 nm	100 uL	30077
CellBrite™ NIR770 Cytoplasmic Membrane Dye	767/806 nm	100 uL	30078
CellBrite™ NIR790 Cytoplasmic Membrane Dye	786/820 nm	100 uL	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. No.
MitoView™ 720	720/798 nm	50 ug	70068-T
		20 x 50 ug	70068