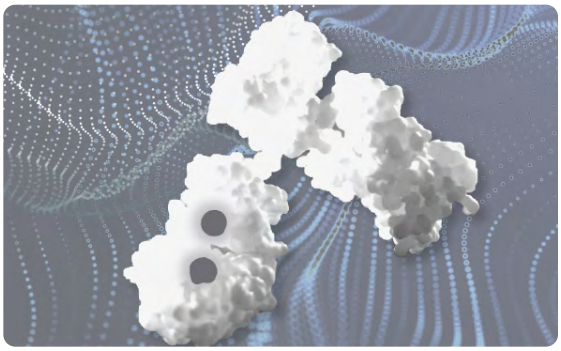
Near-IR Dyes

For *In Vivo* Imaging and NIR Western



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

Near-infrared (also called near-IR or NIR) offer greater sensitivity and specificity than visible light dyes due to low autofluorescence in biological samples at NIR wavelengths. Their strong tissue penetration makes them ideal for *in vivo* imaging, and they enable highly sensitive multiplex Western and In-Cell Western™ assays.

Near-IR dyes are typically large, poorly water-soluble aromatic molecules often modified with negatively charged sulfonate groups to enhance solubility. These charges can shift antibody isoelectric points, increasing non-specific binding (see Fig. 5), and raise immunogenicity *in vivo*. Many also use cyanine backbones, which are less photostable than rhodamine dyes at shorter wavelengths.

Biotium solved common near-IR dye issues by developing near-IR CF® Dyes modified with neutral, water-soluble PEG groups. This improves solubility, reduces the need for sulfonates, shields residual charges, and allows higher dye-to-protein labeling for brighter conjugates (Figs. 2–4) without compromising specificity (Fig. 5). Pegylated CF® Dyes are also brighter, more stable, and less immunogenic *in vivo* (Fig. 10). Biotium's rhodamine-based CF®710, CF®725, and CF®740 dyes offer superior photostability over cyanine dyes.

Additionally, CF® Dye succinimidyl and TFP esters provide much higher labeling efficiency due to high solubility and reactivity (generally >95%)

Industry-Leading Near-IR Dyes

- Brighter and more photostable compared to other near-IR dyes
- Near-IR conjugates with superior signal-to-noise
- Options with either cyanine or rhodamine dye backbone
- 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 equipped with near-IR laser lines and detectors

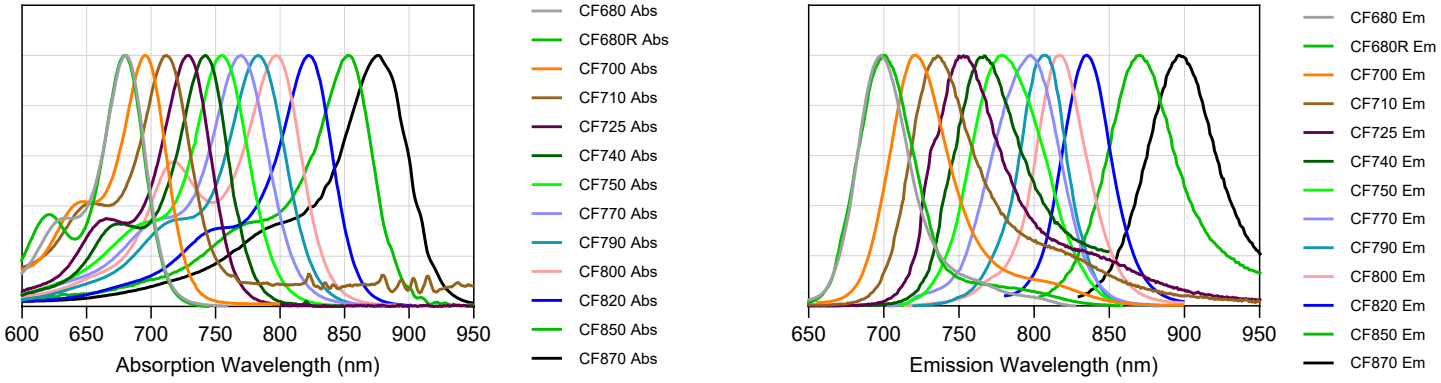


Figure 1. Absorption and emission spectra of near-IR CF® Dyes.

Near-IR CF® Dye Advantages

Exceptionally Bright Conjugates

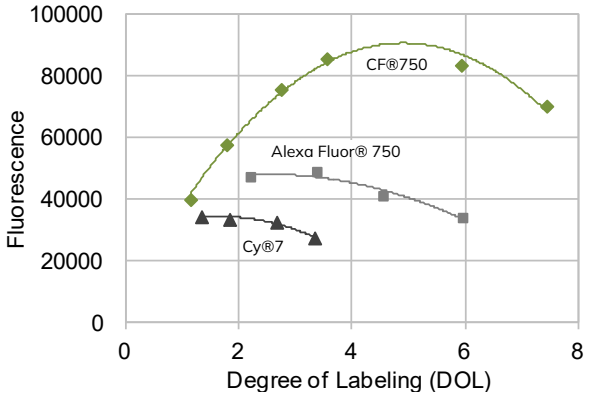


Figure 2. Relative fluorescence of goat anti-mouse IgG conjugates labeled with CF®750, Alexa Fluor® 750, or Cy®7. Because CF®750 is highly water-soluble, a higher degree of labeling (number of dyes per protein) can be achieved without fluorescence quenching, resulting in brighter conjugates.

Higher Signal for NIR Western on LICORbio™ Odyssey®

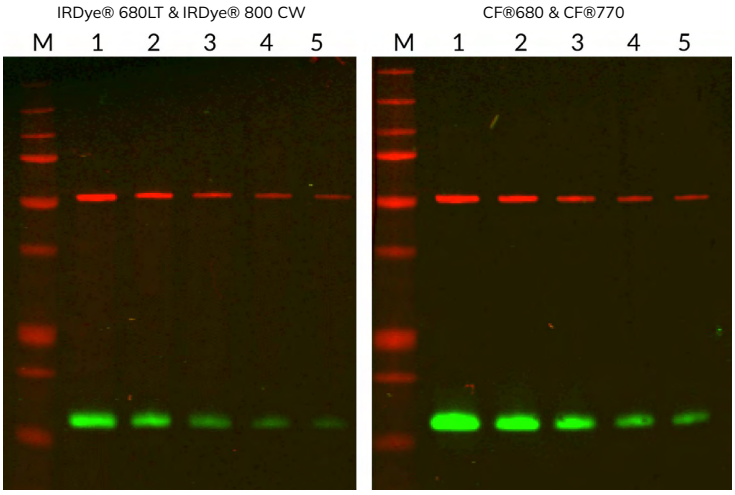


Figure 3. CF® Dye conjugates are brighter than IRDye® conjugates for NIR western. Western blotting of HeLa cell lysate (2 ug to 0.125 ug, lanes 1-5) for tubulin and COX IV, detected by IRDye®680LT or CF®680 (red) and IRDye® 800CW or CF®770 (green) secondary antibodies, respectively. Quantitation of bands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

Highly Water Soluble

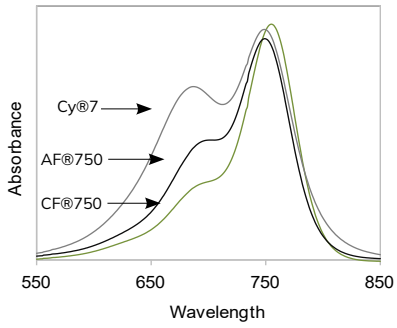


Figure 4. Normalized absorbance spectra of goat anti-mouse IgG labeled with CF®750, Alexa Fluor® 750 (AF®750) or Cy®7. Cy®7 and Alexa Fluor® 750 have large shoulder peaks (arrows), which are indicative of dye aggregation due to poor solubility. Dye aggregates tend to self-quench and therefore don't contribute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

Superior Conjugate Specificity

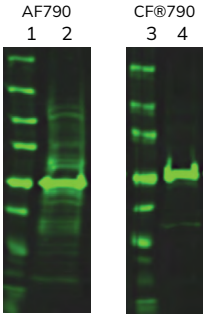


Figure 5. Near-IR CF® Dyes carry less negative charge than other near-IR dyes, resulting in less non-specific binding of conjugates. Western blot detection of tubulin in HeLa cell lysate using secondary antibodies conjugated to Alexa Fluor® 790 (AF790) (lane 2) or CF®790 (lane 4). Lanes 1 and 3 contain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

Remarkably Photostable

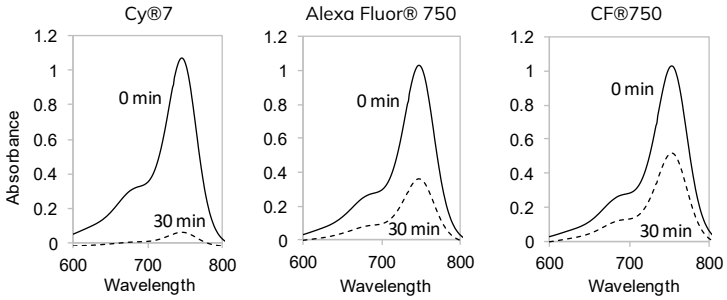


Figure 6. Photostability of Cy®7, Alexa Fluor® 750 and CF®750 dyes. Plots show absorption spectra of the respective dyes before (solid line) and after (dashed line) 30 minutes of sunlight exposure.

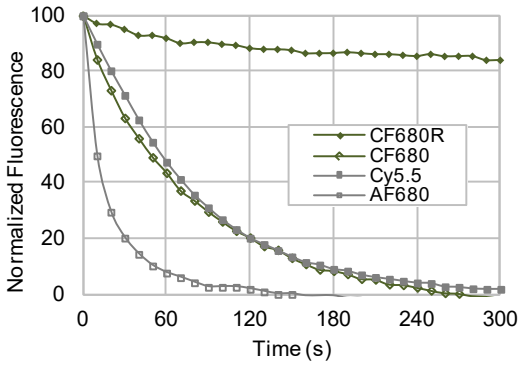


Figure 7. CF®680R is highly photostable. Immunofluorescence was performed for CD3 in Jurkat cells using secondary antibody conjugates of the indicated dyes. Cells were exposed to continuous mercury arc lamp excitation with a Cy®5 filter set. Images were captured every 10 seconds for 5 minutes; mean fluorescence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

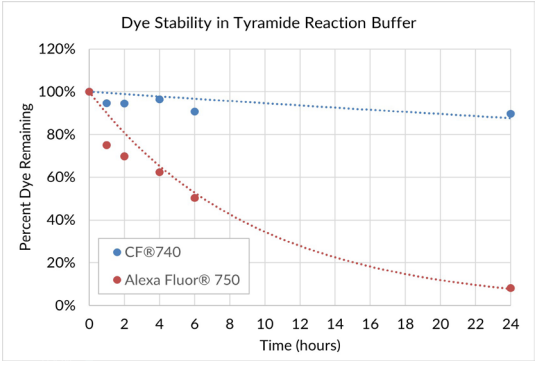


Figure 8. CF®740 and Alexa Fluor® 750 free acids were diluted to 2 µM in Tyramide Amplification Buffer with 0.0015% H_2O_2 and incubated at room temperature, protected from light. CF®740 remained ≥90% stable over 24 hours, while Alexa Fluor® 750 degraded to less than 10% of the starting concentration after 24 hours.

Near-IR CF® Dye Applications

Super-Resolution Microscopy

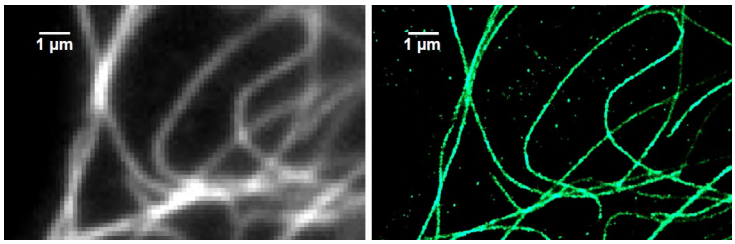


Figure 8. Comparison of microtubule imaging using conventional wide-field microscopy (left) with STORM (right) using CF®680 dye conjugate. Images courtesy of Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

In-Cell Western™ on LICORbio™ Odyssey®

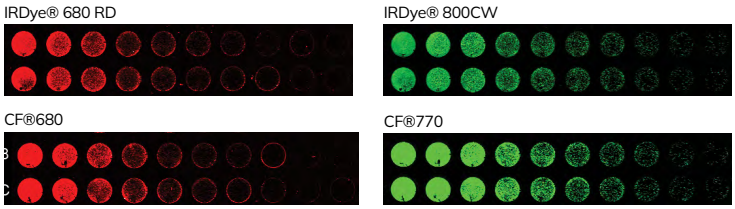


Figure 9. Comparison of CF® Dye and IRDye® secondary antibody conjugates by In-Cell Western™ on the LICORbio™ Odyssey® system. Two-fold dilutions of HeLa cells were grown in 96-well tissue culture plates in duplicate wells. Cells were stained for tubulin and COXIV using CF® Dye or IRDye® secondary antibodies. Near-IR imaging with the LICORbio™ Odyssey® system showed that CF® Dye conjugates produced twice the fluorescence signal of IRDye® conjugates.

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
- Mix-n-Stain™ Antibody & Nanobody Labeling Kits
- Amine-reactive succinimidyl esters and protein labeling kits

In Vivo Imaging

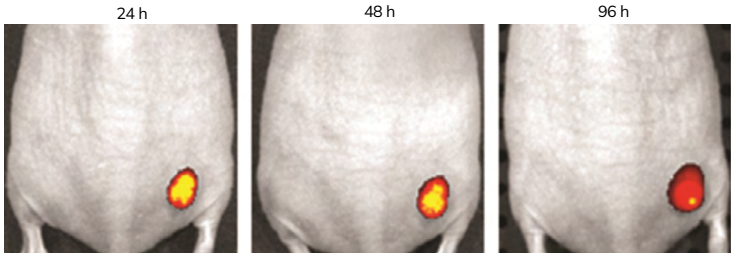


Figure 10. Tumors in mice were imaged using an IVIS® imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin® conjugated to CF®750. Images courtesy of Caliper Life Sciences.

Table 1. Near-Infrared CF® Dyes

Dye	Ex/Em (nm)	Replacement for	Features
CF®680	681/698	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	• CF®680 is the brightest dye among spectrally similar dyes and ideal for protein or antibody labeling • CF®680R offers superior photostability and is suited for labeling small molecules like nucleic acids • Matches 700 channel of LICORbio™ Odyssey® and Atlas™ • CF®680 is validated for multicolor 3D STORM with CF®568, CF®647, and CF®660C • CF®680R is validated for STED, single-molecule spectroscopy, and multi-color STORM
CF®680R	680/701	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	
CF®700	695/720	Alexa Fluor® 700, DyLight® 700, BD Horizon™ Red 718, Spark Red™ 718	• Exceptionally bright and more photostable than other near-IR dyes • Highly water soluble without bearing excessive charge • CF®740 is stable in oxidizing conditions for tyramide signal amplification • CF®750 is validated in super-resolution imaging by STORM • Compatible with LICORbio™ Odyssey® and Atlas™; CF®770 matches the 800 channel
CF®710	712/736	Alexa Fluor® 700	
CF®725	729/750	Unique near-IR dye	
CF®740	742/767	Alexa Fluor® 750, Cy®7, DyLight® 750, IRDye® 750	
CF®750	755/777	Alexa Fluor® 750, Cy®7, DyLight® 750, IRDye® 750	
CF®770	770/797	DyLight® 800, IRDye® 800CW	
CF®790	784/806	Alexa Fluor® 790	
CF®800	797/816	Spectrally similar to Indocyanine Green	
CF®820	822/835	DY-820	
CF®850	852/870	Unique dye	• CF®850 and CF®870 are unique near-IR dyes designed for the 808 nm laser
CF®870	876/896	Unique dye	

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

Product	Ex/Em (nm)	Cat. No.
CF®680 Succinimidyl Ester	681/698	92139
CF®680R Succinimidyl Ester	680/701	92107
CF®700 Succinimidyl Ester	695/720	96067
CF®710 Succinimidyl Ester	712/736	96125
CF®725 Succinimidyl Ester	729/750	96126
CF®740 Succinimidyl Ester	742/767	96109
CF®750 Succinimidyl Ester	755/777	92142
CF®770 Succinimidyl Ester	770/797	92150
CF®790 Succinimidyl Ester	784/806	92155
CF®800 Succinimidyl Ester	797/816	92127
CF®820 Succinimidyl Ester	822/835	96068
CF®850 TFP Ester	852/870	96094
CF®870 TFP Ester	876/896	96095
CF®680 SE Protein Labeling Kit	681/698	92220
CF®680R SE Protein Labeling Kit	680/701	92226
CF®750 SE Protein Labeling Kit	755/777	92221
CF®770 SE Protein Labeling Kit	770/797	92222
VivoBrite™ CF®680 Antibody Labeling Kit	681/698	92160
VivoBrite™ CF®750 Antibody Labeling Kit	755/777	92161
VivoBrite™ CF®770 Antibody Labeling Kit	770/797	92162
VivoBrite™ CF®790 Antibody Labeling Kit	784/806	92163

Other Reactive Dyes for Labeling Thiols, Carbonyls, Click Chemistry, and More

Biotium also offers other CF® Dye reactive formats for labeling carbonyl and thiol groups, as well as azide, BCN, and other click chemistry formats.

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

Label any IgG 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®700	92425	92426	92427
	CF®710	92579	92580	92581
	CF®725	92582	92583	92584
	CF®740	92576	92577	92578
	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

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)	Cat. No.
NucSpot® 680/700, 1000X in DMSO	683/707	41035-T
NucSpot® 750/780, 1000X in DMSO	757/780	41038-T

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)	Cat. No.
CF®680 Annexin V	681/698	29007
CF®680R Annexin V	699/737	29070
CF®700 Annexin V	699/737	29082
CF®750 Annexin V	755/777	29006
CF®770 Annexin V	770/797	29046
CF®790 Annexin V	784/806	29047
CF®800 Annexin V	797/816	29078

Novel Near-IR Membrane Dyes

Biotium carries a number of unique near-IR membrane dyes designed to fit a variety of workflows. This includes ready-to-use lipophilic CellBrite® NIR Cytoplasmic Membrane Dyes, CellBrite® Steady 685 for multi-day imaging of live cells, as well as CytoLiner™ 680/710 and 785/815 for uniform membrane labeling in PFA-fixed cells.

Product	Ex/Em (nm)	Cat. No.
CellBrite® NIR Cytoplasmic Membrane Dyes	Multiple	30070... 30079
CellBrite® Steady 685 Membrane Staining Kit	686/708	30109-T
CytoLiner™ 680/710 Fixed Cell Membrane Stain	682/707	30135-T
CytoLiner™ 785/815 Fixed Cell Membrane Stain	787/819	30140-T

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 (nm)	Cat. No.
MitoView™ 720	720/798	70068-T

Primary Antibodies, Single-Domain VHH, & Secondary Antibody Conjugates

Visit biotium.com to browse our wide selection of primary antibodies, single-domain VHH MiniMab™ antibodies, anti-tag antibodies and secondary antibodies conjugated to our near-IR CF® Dyes, including single-label secondary antibodies optimized for STORM imaging.

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.

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