

# HiLyte Fluor™

Be Brilliant



2nd edition

# Table of Contents

## HiLyte Fluor™ Labeling Dyes

Superior Fluorescent Labeling Dyes Spanning the Full Visible Spectrum.....	1
HiLyte Fluor™ 405 Dye, an Excellent Alternative to Alexa Fluor® 405 & DyLight® 405.....	2
HiLyte Fluor™ 488 Dye, an Excellent Alternative to FITC.....	3
HiLyte Fluor™ 555 Dye, an Excellent Alternative to Cy3™ Dye.....	4
HiLyte Fluor™ 594 Dye, an Excellent Alternative to Texas Red®.....	5
HiLyte Fluor™ 647 Dye, an Excellent Alternative to Cy5™ Dye.....	6
HiLyte Fluor™ 680 Dye, an Excellent Alternative to Cy5.5™ Dye.....	7
HiLyte Fluor™ 750 Dye, an Excellent Alternative to Cy7™ Dye.....	8
AnaTag™ HiLyte Fluor™ Protein Labeling Kits.....	9
HiLyte Fluor™ Labeled Primary and Secondary Antibodies.....	Back Cover
HiLyte Fluor™ Labeled Streptavidins.....	Back Cover

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Alexa Fluor®, Texas Red® (Invitrogen)  
Cy™ (GE Healthcare)  
DyLight Fluor® (Pierce)

## Superior Fluorescent Labeling Dyes Spanning the Full Visible Spectrum

HiLyte Fluor™ dyes, AnaSpec's patented dyes are a series of excellent fluorescent labeling dyes that span the full visible spectrum. For most of the HiLyte Fluor™ dyes, their hydrophilic property makes conjugations to protein easier, minimizing the use of organic solvents. The resulting conjugates are relatively resistant to precipitation during storage. The HiLyte Fluor™ dyes also have better labeling performance than the classic fluorescent labeling dyes such as FITC and rhodamines. Other features of the HiLyte Fluor™ dyes include:

- HiLyte Fluor™ dyes are available in a variety of reactive forms.
- HiLyte Fluor™ conjugates exhibit more intense fluorescence than other spectrally similar conjugates of classic fluorescent dyes such as FITC, TAMRA and ROX under similar excitations.
- HiLyte Fluor™ dyes are more photostable than the classic fluorescent dyes such as FITC, Cy3™ and Cy5™.
- HiLyte Fluor™ dyes have absorption spectra that match the principal output wavelengths of common excitation sources such as 488 nm, 555 nm, 633 nm and 647 nm.
- HiLyte Fluor™ dyes and their conjugates are available in several distinct fluorescent colors.
- HiLyte Fluor™ dyes are highly fluorescent over a broad pH range with little pH sensitivity.

HiLyte Fluor™ dyes are the products of our R&D efforts. Additionally, AnaSpec is rapidly expanding our product lines to meet research needs. We have been developing dyes to solve various limitations with the existing fluorescent labeling reagents while offering classic fluorescent labeling reagents which have high purity and competitive pricing. We also provide custom synthesis and contract research services to meet your special needs. Our strong expertise in both the chemistry of fluorescent dyes and peptides equip us to provide quality services with competitive prices.

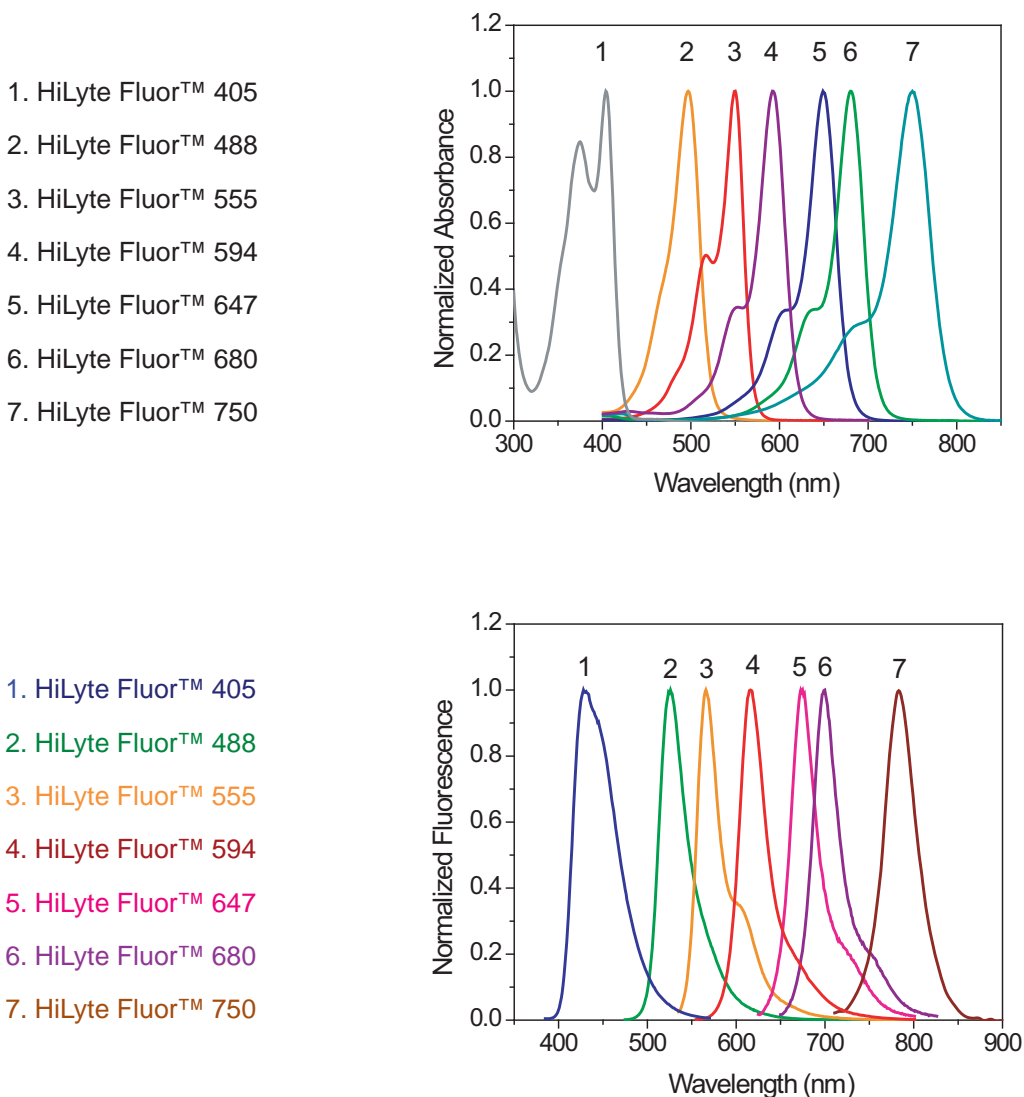


Figure 1. Absorption and emission spectra of AnaSpec's patented HiLyte Fluor™ dyes.

## HiLyte Fluor™ 405 Dye, an Excellent Alternative for Alexa Fluor® 405 and DyLight® 405

HiLyte Fluor™ 405, available in a variety of reactive forms, such as amine-reactive, thiol-reactive and amine-containing derivatives, allows for easy conjugation to biopolymers. HiLyte Fluor™ 405 has spectral characteristics similar to those of Alexa Fluor® 405 and DyLight® 405 dyes with excitation and emission wavelength at 404/428 nm in PBS buffer (pH 7.4). Conjugates of HiLyte Fluor™ 405 are well excited by the 407 nm spectral line of the Krypton laser and the 408 nm line of the violet laser diode. HiLyte Fluor™ 405, whether conjugated to primary tag, secondary antibodies or streptavidin, are prepared in an optimized fluorophore/protein labeling ratio to ensure high fluorescent signal and uncompromised biological function. Extinction coefficient is 35,000 M<sup>-1</sup>cm<sup>-1</sup>, with a quantum yield of 0.54.

Product	Size	Cat#
HiLyte Fluor™ 405 acid	10 mg	89316
HiLyte Fluor™ 405 amine, TFA salt	1 mg	89318
HiLyte Fluor™ 405 C2 maleimide	1 mg	89320
HiLyte Fluor™ 405 hydrazide	1 mg	89319
HiLyte Fluor™ 405 succinimidyl ester (SE)	1 mg 5 mg	89317-1 89317

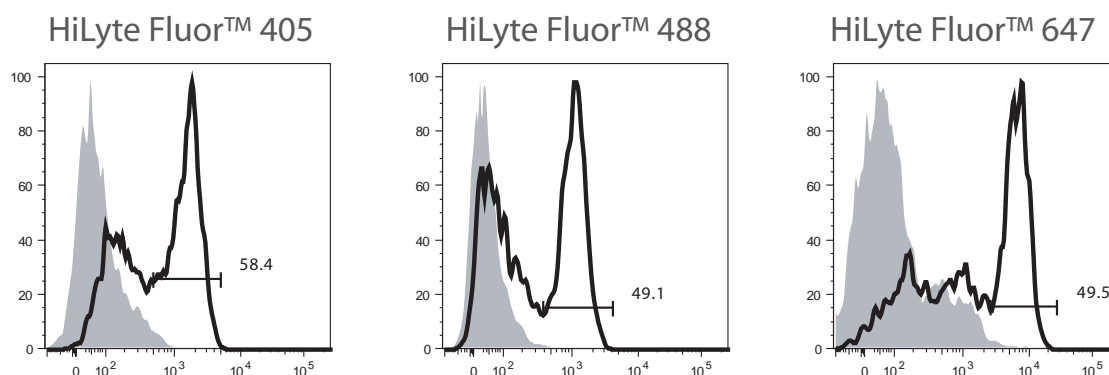
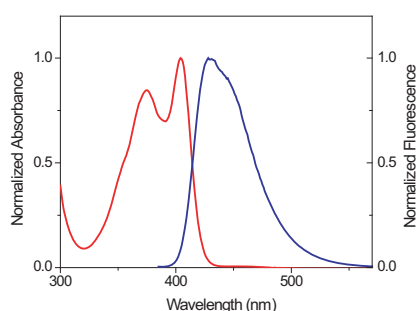


Figure 2. Absorption and emission spectra of HiLyte Fluor™ 405 (top panel). Flow cytometry results are shown in the bottom panel. Human PBMC cells were stained with anti-CD3 mAb (0.9 mg/ml) at 1:500 dilution. Secondary antibodies, goat anti-mouse IgG labeled with HiLyte Fluors at 0.5 mg/ml (cat# 61057-H405, 61057-H488, 61057-H647) were used at 1:200 dilution. Filled histogram: secondary antibody staining only control.

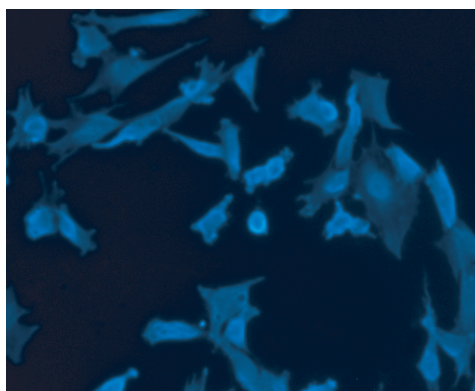


Figure 3. Alpha-tubulin in 3T3 cells probed with mouse anti- $\alpha$  tubulin antibody, visualized with HiLyte Fluor™ 405 conjugated goat anti-mouse IgG (Cat# 28176-H405).

## HiLyte Fluor™ 488 Dye, an Excellent Alternative for FITC

Although FITC is still the most popular fluorescent labeling dye for preparing green fluorescent bioconjugates, there are limitations, such as severe photobleaching for microscope imaging and pH-sensitive fluorescence. Protein conjugates prepared with HiLyte Fluor™ 488 dyes (Ex/Em=497/525 nm) are far more superior compared to conjugates of fluorescein derivatives such as FITC (Figure 2). HiLyte Fluor™ 488 conjugates are significantly brighter than fluorescein conjugates and are much more photostable. Extinction coefficient is 70,000 M<sup>-1</sup>cm<sup>-1</sup>, with a quantum yield of 0.91. Additionally, the fluorescence of HiLyte Fluor™ 488 is not affected by pH (4-10). This pH insensitivity property is a major improvement over fluorescein, which emits maximum fluorescence only at pH above 9.

Product	Size	Cat#
HiLyte Fluor™ 488 acid	10 mg	81160
HiLyte Fluor™ 488 amine, TFA salt	1 mg	81162
HiLyte Fluor™ 488 C2 maleimide	1 mg	81164
HiLyte Fluor™ 488 hydrazide	1 mg	81163
HiLyte Fluor™ 488 succinimidyl ester (SE)	1 mg 5 mg	81161-1 81161

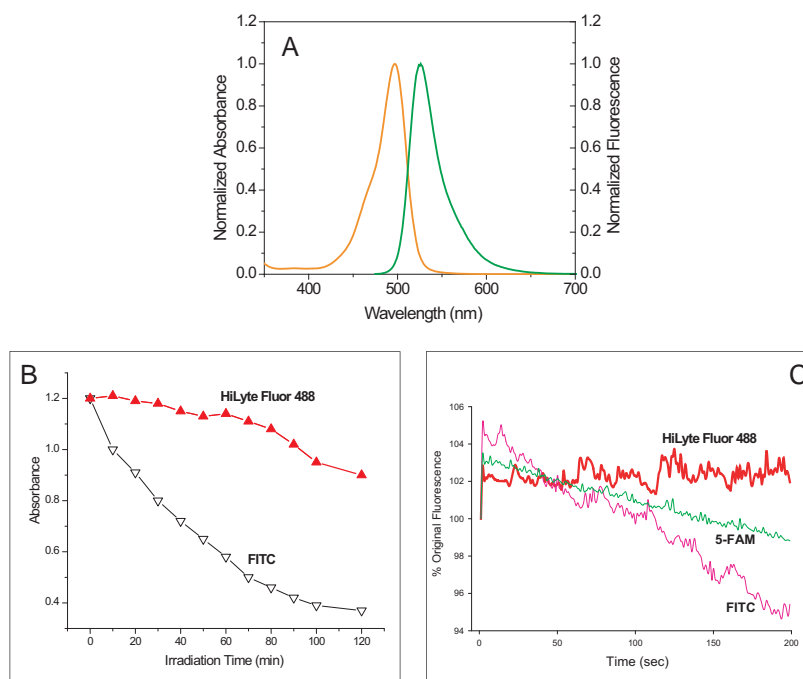


Figure 4. Absorption and emission spectra of HiLyte Fluor™ 488 (panel A). Panels B and C show the photostability of HiLyte Fluor™ 488 compared to FITC and FAM.

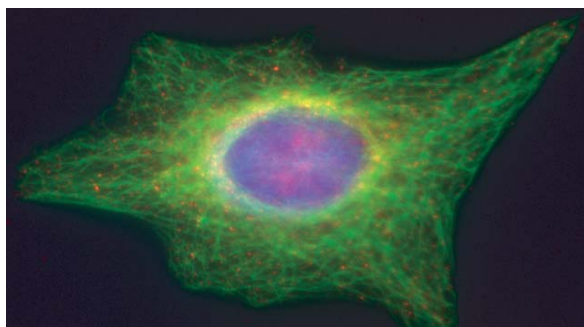


Figure 5. Tubulin in human glioblastoma cells was probed with mouse anti-tubulin, visualized with HiLyte Fluor™ 488-conjugated rabbit anti-mouse IgG (Cat# 28164-H488). Nuclei were stained with Hoechst 33342 (Cat# 83218).

## HiLyte Fluor™ 555 Dye, an Excellent Alternative for Cy3™ Dye

Although Cy3™ is the preferred dye for preparing orange fluorescent bioconjugates, HiLyte Fluor™ 555 conjugates are more photostable and brighter. Compared to the spectra of Cy3™ dyes, the spectra of HiLyte Fluor™ 555 conjugates are slightly red-shifted, resulting in an optimal match of filters designed for Cy3™ dyes. Extinction coefficient is 150,000 M<sup>-1</sup>cm<sup>-1</sup>, with a quantum yield of 0.10. The photostability of HiLyte Fluor™ 555 provides researchers with additional time for image capture.

Product	Size	Cat#
HiLyte Fluor™ 555 acid	5 mg	81250
HiLyte Fluor™ 555 amine	1 mg	81252
HiLyte Fluor™ 555 C2 maleimide	1 mg	81254
HiLyte Fluor™ 555 hydrazide	1 mg	81253
HiLyte Fluor™ 555 succinimidyl ester (SE)	1 mg	81251

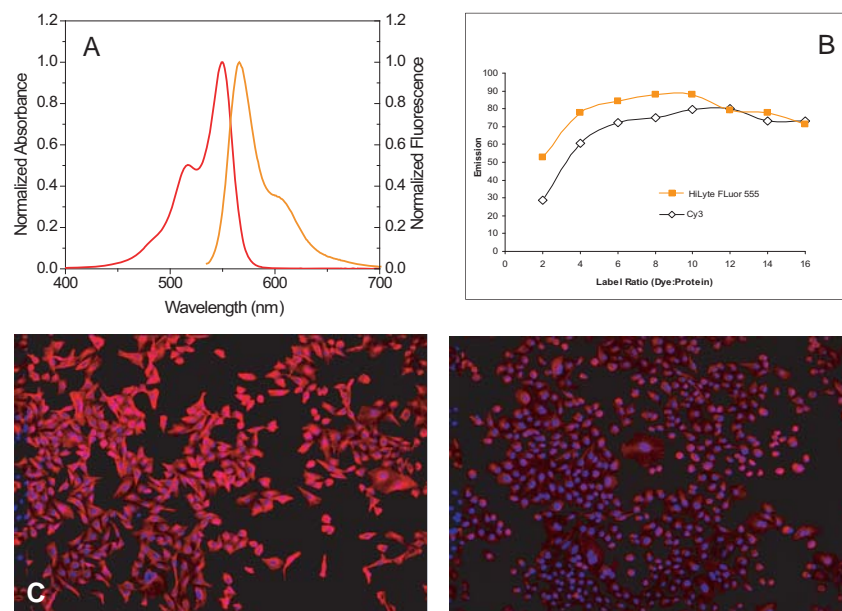


Figure 6. Absorption and emission spectra of HiLyte Fluor™ 555 (panel A). Comparison data of HiLyte Fluor™ 555 and Cy3™ emission at different dye to protein ratio (panel B). 3T3 cells incubated with anti-tubulin antibody and goat-anti-rabbit antibodies, labeled either with HiLyte Fluor™ 555 (panel C), or Cy3™ (panel D). Nuclei were stained with Hoechst 33342 (Cat# 83218).

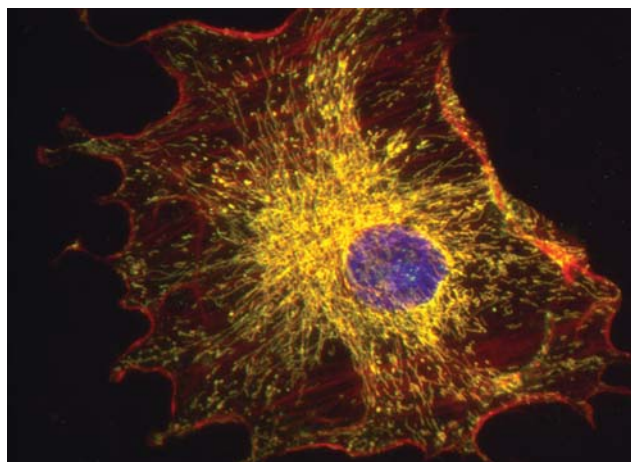


Figure 7. Bovine pulmonary artery endothelial cells actins were stained with biotin-conjugated phalloidin, visualized with HiLyte Fluor™ 555 conjugated streptavidin (Cat# 60666). Mitochondria were stained with mouse anti-Oxphos complex V, visualized with HiLyte Fluor™ 488-conjugated goat anti-mouse (Cat# 28175-H488), nuclei labeled with Hoechst 33342 (Cat# 83218).

## HiLyte Fluor™ 594 Dye, an Excellent Alternative for Texas Red®

HiLyte Fluor™ 594 has spectral characteristics similar to those of Texas Red®, with excitation and emission wavelength at 593/615 nm in PBS buffer (pH 7.4), and 596/617 nm when conjugated to macromolecules (see spectra below). The labeling performance and stability properties of HiLyte Fluor™ 594 are better than those of Texas Red®. The higher extinction coefficient (80,000 M<sup>-1</sup>cm<sup>-1</sup>) and a lower correction factor (0.17) of HiLyte Fluor™ 594 are better than those of Alexa Fluor™ 594. The fluorescence quantum yield of HiLyte Fluor™ 594 is 0.9 in aqueous solution. HiLyte Fluor™ 594 conjugated streptavidin provides high fluorescence intensity and low background as validated in immunofluorescence staining of mammalian cells. Biomolecules conjugated to HiLyte Fluor™ 594 exhibit little spectral overlap with green-fluorescent conjugates, and can be efficiently excited by 568 nm line of Ar-Kr laser and by the 594 nm line of orange He-Ne laser.

Product	Size	Cat#
HiLyte Fluor™ 594 acid	10 mg	81271
HiLyte Fluor™ 594 amine TFA salt	1 mg	81273
HiLyte Fluor™ 594 C2 maleimide	1 mg	81275
HiLyte Fluor™ 594 hydrazide	1 mg	81253
HiLyte Fluor™ 594 succinimidyl ester (SE)	1 mg 5 mg	81272-1 81272-5

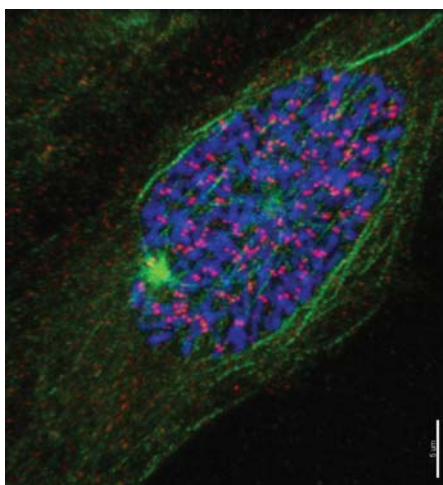
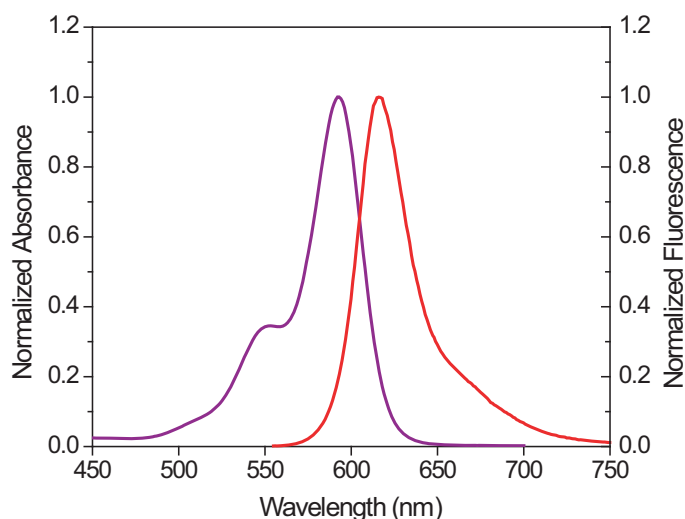


Figure 8. Absorption and emission spectra of HiLyte Fluor™ 594 (top). Bottom image shows  $\alpha$ -tubulin in 3T3 cells probed with mouse anti- $\alpha$ -tubulin and visualized with HiLyte Fluor™ 488 conjugated goat anti-mouse IgG (Cat# 28175-H488), anti-kinetochore protein Zwint10 visualized with HiLyte Fluor™ 594 conjugated goat anti-rabbit IgG (Cat# 28175-H594); nuclei were stained with DAPI. Image courtesy of Dr. Xue-Jun Sun, Cross Cancer Institute, Edmonton, Alberta, Canada.

## HiLyte Fluor™ 647 Dye, an Excellent Alternative for Cy5™ Dye

Cy5™ is the preferred dye for preparing near-infrared fluorescent bioconjugates. Compared to the spectra of Cy5™ dyes, the spectra of HiLyte Fluor™ 647 conjugates are only slightly red-shifted. This slight change in absorption spectrum makes HiLyte Fluor™ 647 dyes an optimal match of filters designed for Cy5™ dyes. In a side-by-side comparison of antibody conjugates of HiLyte Fluor™ 647 dyes and Cy5™ conjugates (supplied by other companies), the total fluorescence of HiLyte Fluor™ 647 labeled secondary antibodies is significantly higher than that of Cy5™ conjugates. Extinction coefficient is 250,000 M<sup>-1</sup>cm<sup>-1</sup>, with a quantum yield of 0.33. Unlike Cy5™ dyes, HiLyte Fluor™ 647 dyes have very little change in absorption or fluorescence spectra when conjugated to most proteins, oligonucleotides and nucleic acids, thus yielding greater total fluorescence at the same degree of substitution.

Product	Size	Cat#
HiLyte Fluor™ 647 acid	5 mg	81255
HiLyte Fluor™ 647 amine	1 mg	81257
HiLyte Fluor™ 647 C2 maleimide	1 mg	81259
HiLyte Fluor™ 647 hydrazide	1 mg	81258
HiLyte Fluor™ 647 succinimidyl ester (SE)	1 mg	81256

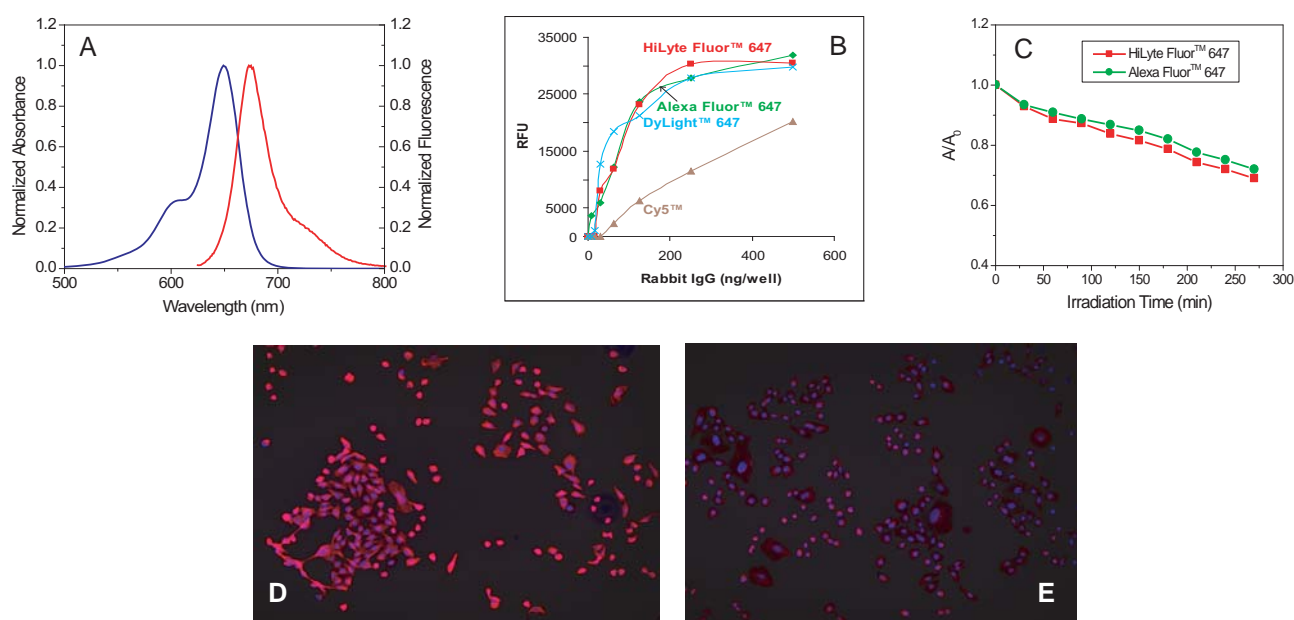


Figure 9. Absorption and emission spectra of HiLyte Fluor™ 647 (panel A). Performance of dye-goat anti-rabbit IgG conjugates (panel B). HiLyte Fluor™ 647 and Alexa Fluor™ 647 photostability over time (panel C). 3T3 cells incubated with anti-tubulin antibody and goat-anti-rabbit antibodies, labeled either with HiLyte Fluor™ 647 (panel D), or Cy5™ (panel E), nuclei were stained with Hoechst 33342 (Cat# 83218).

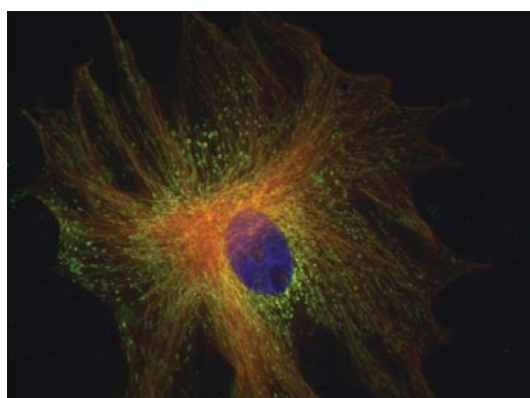


Figure 10.  $\alpha$ -Tubulins of bovine pulmonary artery endothelial cells were probed with biotin-conjugated mouse anti- $\alpha$ -tubulin, visualized with HiLyte Fluor™ 647 conjugated streptavidin (Cat# 60667). Mitochondria were detected with mouse anti-Oxphos V complex, visualized with HiLyte Fluor™ 488-conjugated goat anti-mouse (Cat# 28175-H488), nuclei stained with DAPI (Cat# 83210).



## HiLyte Fluor™ 680 Dye, an Excellent Alternative for Cy5.5™ Dye

With a peak excitation at 678 nm and emission maximum at 699 nm, HiLyte Fluor™ 680 dyes are spectrally similar to Cy5.5™ dyes. Fluorescence emission of HiLyte Fluor™ 680 dyes is well separated from that of other commonly used red fluorophores, such as TAMRA, R-phycoerythrin and HiLyte Fluor™ 647 dyes, making it ideal for three and four-color labeling. Extinction coefficient is  $190,000 \text{ M}^{-1}\text{cm}^{-1}$ , with a quantum yield of 0.36.

Product	Size	Cat#
HiLyte Fluor™ 680 acid	5 mg	81260
HiLyte Fluor™ 680 amine	1 mg	81262
HiLyte Fluor™ 680 C2 maleimide	1 mg	81264
HiLyte Fluor™ 680 hydrazide	1 mg	81263
HiLyte Fluor™ 680 succinimidyl ester (SE)	1 mg	81261

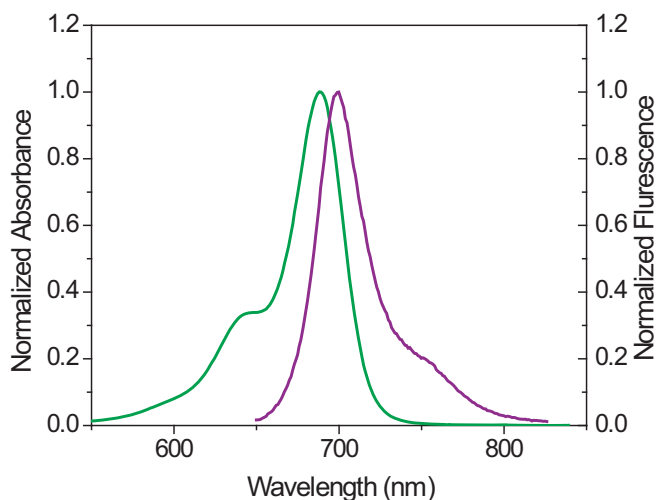


Figure 11. Absorption and emission spectra of HiLyte Fluor™ 680.

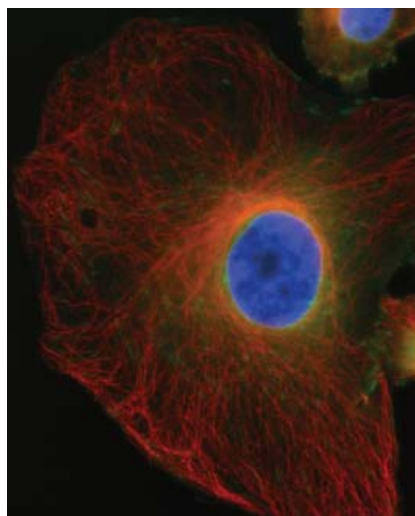


Figure 12. CHO-K1 cells were labeled with rabbit anti-tubulin primary antibody and a secondary antibody goat anti-rabbit IgG conjugated to HiLyte Fluor™ 680 (red); Paxillin EGFP (green) and a nuclear stain, DAPI (blue). Image courtesy of Kimberly Young and Claire M. Brown, McGill University Imaging Facility, Montreal, Canada.

## HiLyte Fluor™ 750 Dye, an Excellent Alternative for Cy7™ Dye

Spectrally similar to Cy7™ dye, HiLyte Fluor™ 750 dye is the longest-wavelength HiLyte Fluor™ dye currently available. Its fluorescence emission maximum at 778 nm is well separated from commonly used far-red fluorophores, including HiLyte Fluor™ 647, HiLyte Fluor™ 680 or allophycocyanin (APC), facilitating multicolor analysis. Extinction coefficient is 275,000  $M^{-1}cm^{-1}$ , with a quantum yield of 0.12. With a peak excitation at ~753 nm, conjugates of HiLyte Fluor™ 750 dyes can be excited by a xenon-arc lamp or dye-pumped lasers operating in the 720–750 nm range.

Product	Size	Cat#
HiLyte Fluor™ 750 acid	5 mg	81265
HiLyte Fluor™ 750 amine	1 mg	81267
HiLyte Fluor™ 750 C2 maleimide	1 mg	81269
HiLyte Fluor™ 750 hydrazide	1 mg	81268
HiLyte Fluor™ 750 succinimidyl ester (SE)	1 mg	81266

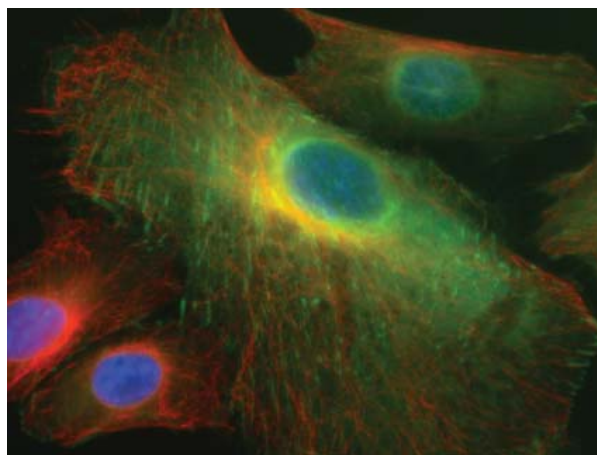
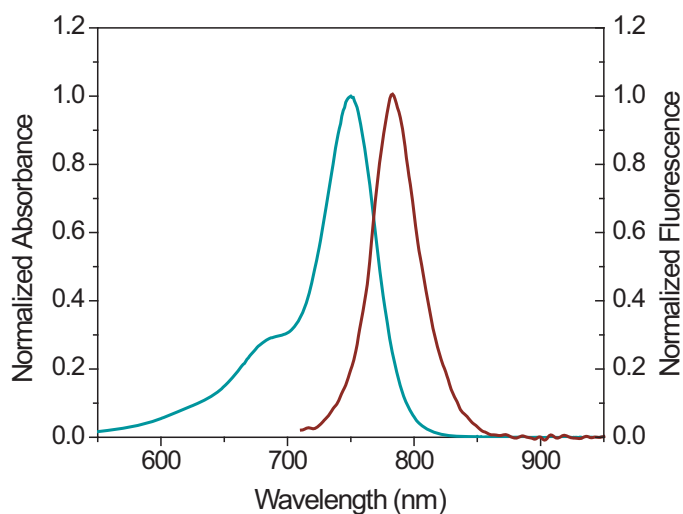


Figure 13. Absorption and emission spectra of HiLyte Fluor™ 750 (top). Bottom image shows an image of CHO-K1 cells labeled with rabbit anti-tubulin primary antibody and a secondary antibody goat anti-rabbit IgG conjugated to HiLyte Fluor™ 750 (red); Paxillin EGFP (green) and a nuclear stain, DAPI (blue). Image courtesy of Kimberly Young and Claire M. Brown, McGill University Imaging Facility, Montreal, Canada.

## AnaTag™ Protein Labeling Kits

The AnaTag™ HiLyte Fluor™ Protein Labeling Kits provide a convenient way to label proteins using the succinimidyl ester (SE) reactive form of the HiLyte Fluor™ dyes. The succinimidyl ester shows good reactivity and selectivity with aliphatic amines of the protein and forms a carboxamide bond, which is identical to, and is as stable as the natural peptide bond. HiLyte Fluor™-protein conjugates may be used in immunofluorescent staining, fluorescent in situ hybridization, flow cytometry and other biological applications. Each kit has all the essential components for performing the conjugation reaction and for purifying the HiLyte Fluor™-protein conjugate.

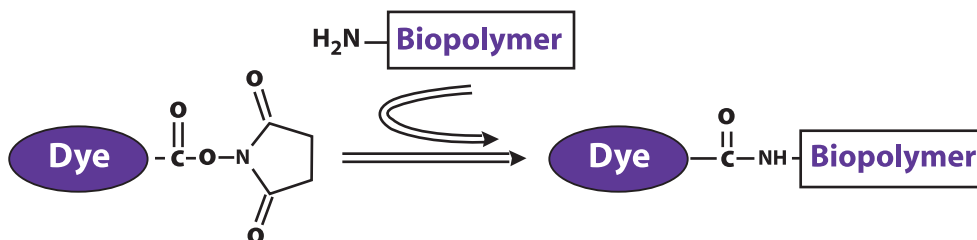


Figure 14. The succinimidyl ester (SE) group of the fluorophore reacts with the amino group of lysines on the protein to form a stable carboxamide bond.

Table 1. AnaTag™ HiLyte Fluor™ Protein Labeling kits are available in large scale (3x5 mg) or imicroscale (3x200 ug) sizes.

Product	Abs / Em (nm / nm)	3 x 5 mg	3 x 200 ug
AnaTag™ HiLyte Fluor™ 405 Protein Labeling Kit	407 / 429	72143	72142
AnaTag™ HiLyte Fluor™ 488 Protein Labeling Kit	499 / 523	72047	72048
AnaTag™ HiLyte Fluor™ 555 Protein Labeling Kit	553 / 568	72045	72046
AnaTag™ HiLyte Fluor™ 594 Protein Labeling Kit	596 / 617	72121	72120
AnaTag™ HiLyte Fluor™ TR Protein Labeling Kit	591 / 622	72051 (3x1mg)	72052
AnaTag™ HiLyte Fluor™ 647 Protein Labeling Kit	652 / 669	72049	72050
AnaTag™ HiLyte Fluor™ 680 Protein Labeling Kit	678 / 699	72119	72118
AnaTag™ HiLyte Fluor™ 750 Protein Labeling Kit	754 / 778	72043	72044

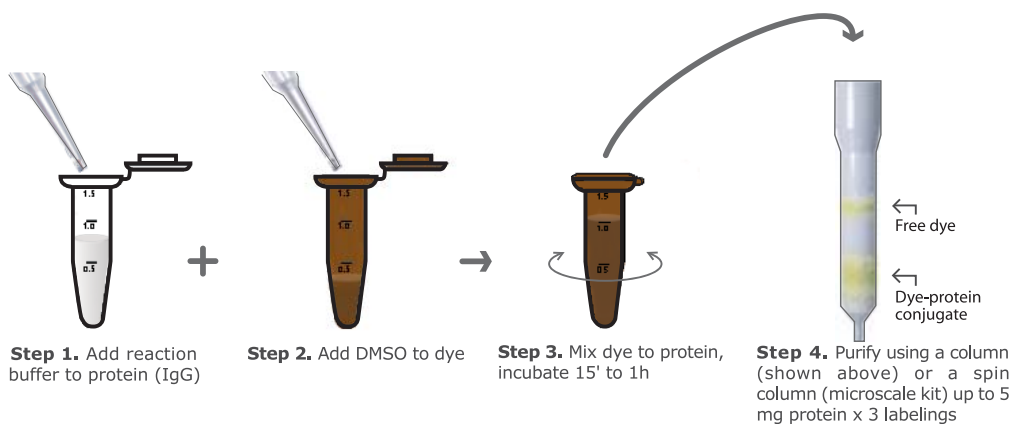


Figure 15. Labeling of an amino group (for instance, side chain of lysine) on a biopolymer with a succinimidyl ester of a dye in 4 easy steps using the AnaTag™ Protein Labeling Kits.

## HiLyte Fluor™ Labeled Primary and Secondary Antibodies

AnaSpec's HiLyte Fluor™ dye-IgG conjugates are prepared using optimal fluorophore to protein labeling ratio to ensure high fluorescent signal and uncompromised IgG function. These HiLyte Fluor™ labeled primary (Table 2) and secondary antibodies (Table 3) can be used in applications such as fluorescence microscopy and flow cytometry. AnaSpec also provides labeling service of our catalog or custom antibodies. Labels include the HiLyte Fluor™ dyes or the classic dyes, such as FITC and TAMRA.

Table 2. Examples of some HiLyte Fluor™ labeled primary antibodies.

Product	HiLyte Fluor™ 405	HiLyte Fluor™ 488	HiLyte Fluor™ 555	HiLyte Fluor™ 594	HiLyte Fluor™ 647
Anti-a-Actin (smooth muscle), polyclonal	29553-H405	29553-H488	29553-H555	29553-H594	29553-H647
Anti-GST Tag, monoclonal	29531-H405	29531-H488	29531-H555	29531-H594	29531-H647
Anti-His Tag, polyclonal	29673-H405	29673-H488	29673-H555	29673-H594	29673-H647
Anti-His Tag, monoclonal	61250-H405	61250-H488	61250-H555	61250-H594	61250-H647

Table 3. Listed below are the catalog numbers of HiLyte Fluor™ dyes labeled secondary antibodies at 1 mg. For 0.5 mg size, simply substitute the middle number of the catalog numbers to 05. For example, 27175-05-H405.

Product	HiLyte Fluor™ 405	HiLyte Fluor™ 488	HiLyte Fluor™ 555	HiLyte Fluor™ 594	HiLyte Fluor™ 647	HiLyte Fluor™ 680	HiLyte Fluor™ 750
Goat anti-mouse IgG (H+L)	28175-1-H405	28175-1-H488	28175-1-H555	28175-1-H594	28175-1-H647	28175-1-H680	28175-1-H750
Goat anti-mouse IgG (H+L), highly cross absorbed	61057-1-H405	61057-1-H488	61057-1-H555	61057-1-H594	61057-1-H647	61057-1-H680	61057-1-H750
Goat anti-rabbit IgG (H+L)	28176-1-H405	28176-1-H488	28176-1-H555	28176-1-H594	28176-1-H647	28176-1-H680	28176-1-H750
Goat anti-rabbit IgG (H+L), highly cross-absorbed	61056-1-H405	61056-1-H488	61056-1-H555	61056-1-H594	61056-1-H647	61056-1-H680	61056-1-H750
Rabbit anti-mouse IgG (H+L)	28164-1-H405	28164-1-H488	28164-1-H555	28164-1-H594	28164-1-H647	28164-1-H680	28164-1-H750
Rabbit anti-goat IgG (H+L)	28168-1-H405	28168-1-H488	28168-1-H555	28168-1-H594	28168-1-H647	28168-1-H680	28168-1-H750
Rabbit anti-chicken IgY (H+L)	29709-1-H405	29709-1-H488	29709-1-H555	29709-1-H594	29709-1-H647	29709-1-H680	29709-1-H750

### HiLyte Fluor™ Labeled Secondary Antibodies Sampler Kits

Table 4. These sampler kits contain 6 different HiLyte Fluor™ labeled secondary antibodies - HiLyte Fluor™ 488, 555, and 647 labeled goat anti-mouse IgGs and goat anti-rabbit IgGs.

Size	Goat anti-mouse IgG Goat anti-rabbit IgG	Goat anti-mouse IgG Goat anti-rabbit IgG (highly cross-adsorbed)	Rabbit anti-mouse IgG Rabbit anti-goat IgG
6 x 5 ug	72002-5	72001-5	72000-5
6 x 50 ug	72002-50	72001-50	72000-50

### HiLyte Fluor™ Streptavidin Conjugates

Table 5. A listing of HiLyte Fluor™-streptavidin conjugates.

Product	HiLyte Fluor™ 405	HiLyte Fluor™ 488	HiLyte Fluor™ 555	HiLyte Fluor™ 594	HiLyte Fluor™ TR	HiLyte Fluor™ 647	HiLyte Fluor™ 680	HiLyte Fluor™ 750
Streptavidin	60672-H405	60665	60666	60672-H594	60671	60667	60659-H680	60659-H750

### HiLyte Fluor™ Labeled Streptavidin Sampler Kits

Table 6. These sampler kits contain streptavidin conjugates of HiLyte Fluor™-488, 555 and 647.

Size	HiLyte Fluor™ Labeled Streptavidin Sampler Kits
3 x 20 ug	72003-20
3 x 200 ug	72003-200