

# 3

## 3.3 Fluorescent dyes & protein labelling kits

205 HiLyte Fluor™ Dyes

211 AnaTag™ Protein labelling kits



# HiLyte Fluor™ Dyes

## Superior fluorescent labelling dyes spanning the full visible spectrum

HiLyte Fluor™ dyes, Eurogentec/AnaSpec's patented dyes are a series of excellent fluorescent labelling 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 labelling performance than the classic fluorescent labelling dyes such as FITC and rhodamines. Other features of the HiLyte Fluor™ dyes:

- 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, we are rapidly expanding our product lines to meet research needs. We have been developing dyes to solve various limitations with the existing fluorescent labelling reagents while offering classic fluorescent labelling 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.

1. HiLyte Fluor™ 488
2. HiLyte Fluor™ 555
3. HiLyte Fluor™ 594
4. HiLyte Fluor™ 647
5. HiLyte Fluor™ 680
6. HiLyte Fluor™ 750

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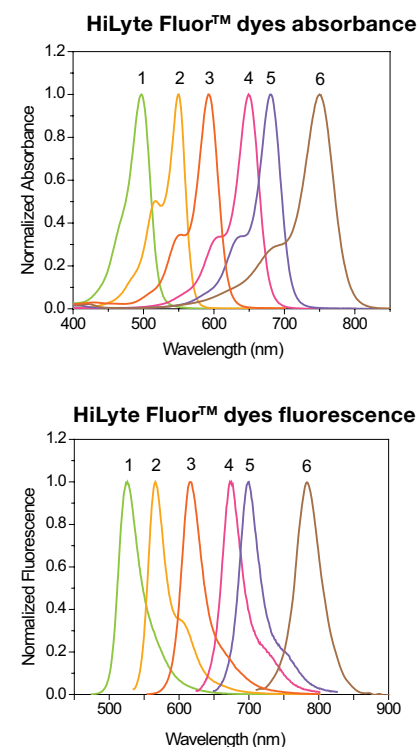


Figure 1. Absorption and emission spectra of AnaSpec's patented HiLyte Fluor™ dyes.

### List of available reactive dyes

Fluorophore	Abs / Em (nm / nm)
AMCA-X	353 / 442
HiLyte Fluor™ 405	404 / 428
5-FITC	494 / 519
5-FAM	495 / 520
HiLyte Fluor™ 488	499 / 523
5 TAMRA	547 / 574
HiLytePlus™ 555	552 / 567
HiLyte Fluor™ 555	553 / 568
5-ROX	573 / 602
HiLyte Fluor™ TR	591 / 622
HiLyte Fluor™ 594	593 / 616
HiLytePlus™ 647	649 / 669
HiLyte Fluor™ 647	652 / 669
HiLyte Fluor™ 680	678 / 699
HiLyte Fluor™ 750	754 / 778

## HiLyte Fluor™ 488 Dye, excellent replacement for FITC

Although FITC is still a popular fluorescent labelling 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 nm/525 nm) are far 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  $70000 \text{ M}^{-1}\text{cm}^{-1}$ , with a quantum yield of 0.91. Additionally, the fluorescence of HiLyte Fluor™ 488 is not affected by pH (4-10). This pH insensitivity is a major improvement over fluorescein, which emits its maximum fluorescence only at pH above 9.

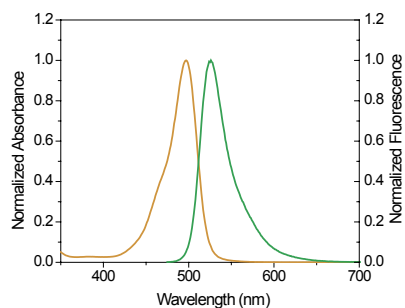


Figure 2. Absorption and emission spectra of HiLyte Fluor™ 488

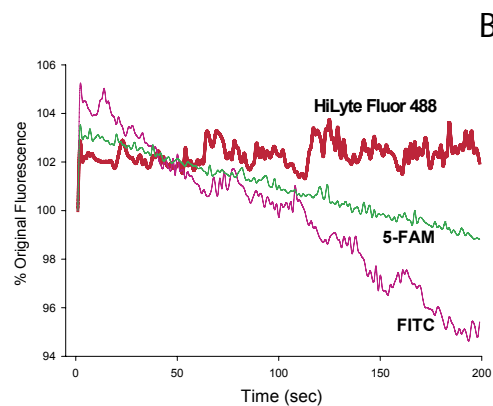
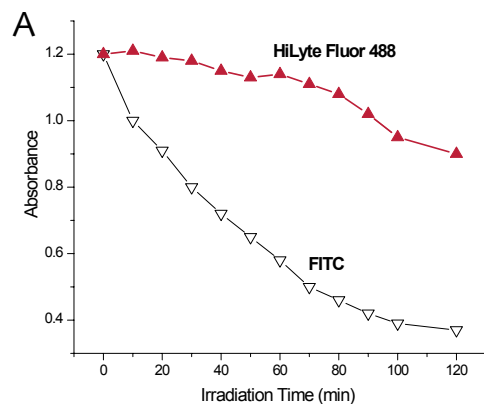


Figure 3. Panels A and B show the photostability of HiLyte Fluor™ 488 compared to FITC and FAM.

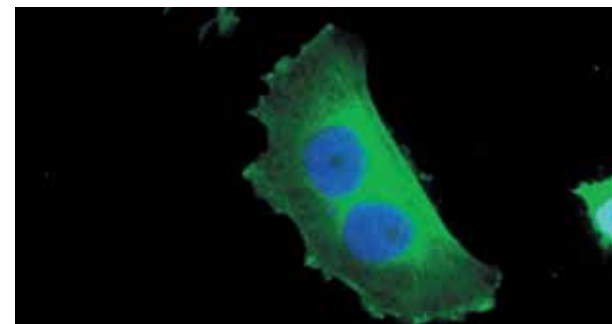


Figure 4. Tubulin in human glioblastoma cells was probed with mouse anti-tubulin, visualized with HiLyte Fluor™ 488-conjugated rabbit anti-mouse IgG (AS-28164-H488). Nuclei were stained with Hoechst 33342 (AS-83218).

### HiLyte Fluor™ 488 Dye

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

## HiLyte Fluor™ 555 Dye, an excellent replacement 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 150000 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.

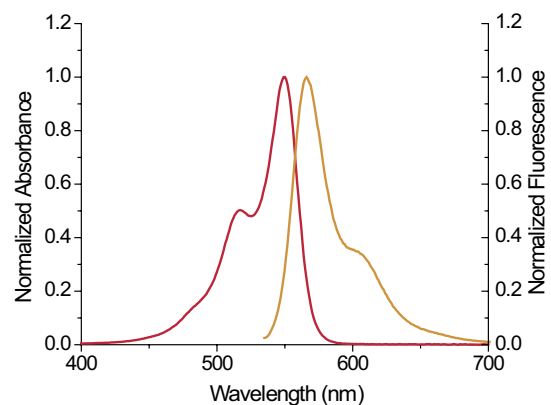


Figure 5. Absorption and emission spectra of HiLyte Fluor™ 555

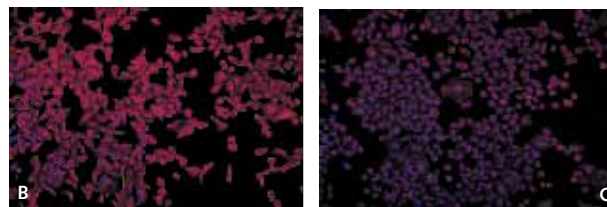
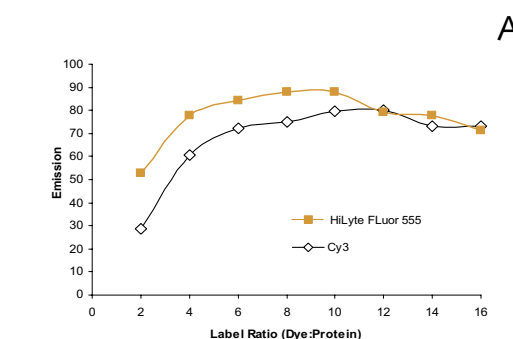


Figure 6. Comparison data of HiLyte Fluor™ 555 and Cy3™ emission at different dye to protein ratio (panel A). 3T3 cells incubated with anti-tubulin antibody and goat-anti-rabbit antibodies, labeled either with HiLyte Fluor™ 555 (panel B), or Cy3™ (panel C). Nuclei were stained with Hoechst 33342 (AS-83218).

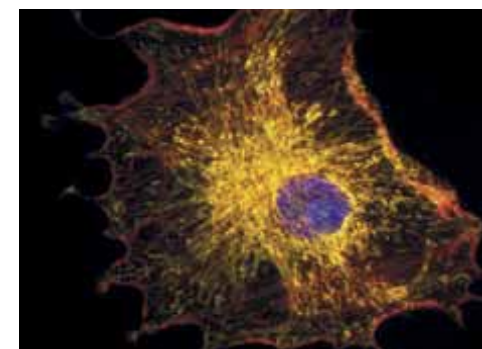


Figure 7. Bovine pulmonary artery endothelial cells actins were stained with biotin-conjugated phalloidin, visualized with HiLyte Fluor™ 555 conjugated streptavidin (AS-60666). Mitochondria were stained with mouse anti-Oxphos complex V, visualized with HiLyte Fluor™ 488-conjugated goat anti-mouse (AS-28175-H488),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

### HiLyte Fluor™ 555 Dye

Product	Size	Reference
HiLyte Fluor™ 555 acid	5 mg	AS-81250
HiLyte Fluor™ 555 amine	1 mg	AS-81252
HiLyte Fluor™ 555 C2 maleimide	1 mg	AS-81254
HiLyte Fluor™ 555 hydrazide	1 mg	AS-89304-1
HiLyte Fluor™ 555 succinimidyl ester (SE)	1 mg	AS-81251

## HiLyte Fluor™ 594 Dye, an excellent replacement 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 labelling performance and stability properties of HiLyte Fluor™ 594 are better than those of Texas Red®. The higher extinction coefficient ( $80000 \text{ M}^{-1}\text{cm}^{-1}$ ) 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.

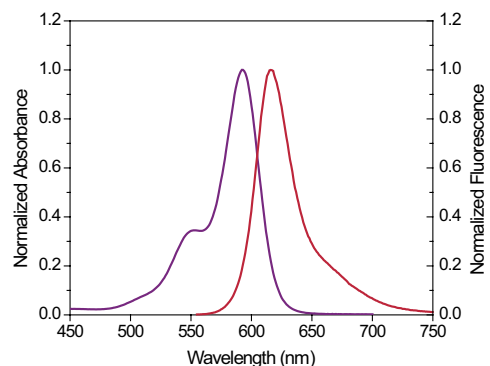


Figure 8. Absorption and emission spectra of HiLyte Fluor™ 594

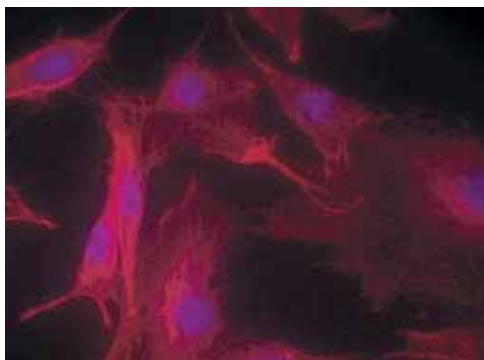


Figure 9. Image shows  $\alpha$ -tubulin in 3T3 cells probed with mouse anti- $\alpha$ -tubulin and visualized with HiLyte Fluor™ 594 conjugated goat anti-mouse IgG (AS-28175-H594), nuclei were stained with DAPI (AS-83210).

### HiLyte Fluor™ 594 Dye

Product	Size	Reference
HiLyte Fluor™ 594 acid	10 mg	AS-81271
HiLyte Fluor™ 594 amine TFA salt	1 mg	AS-81273
HiLyte Fluor™ 594 C2 maleimide	1 mg	AS-81275
HiLyte Fluor™ 594 hydrazide	1 mg	AS-81274
HiLyte Fluor™ 594 succinimidyl ester (SE)	1 mg	AS-81272-1
	5 mg	AS-81272-5

## HiLyte Fluor™ 647 Dye, an excellent replacement 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 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.

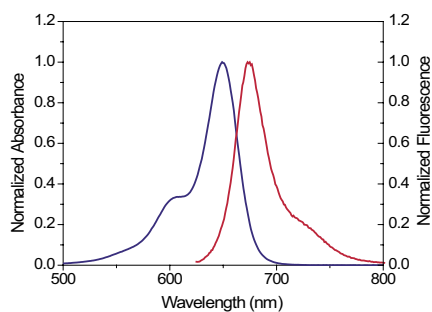


Figure 10 Absorption and emission spectra of HiLyte Fluor™ 647

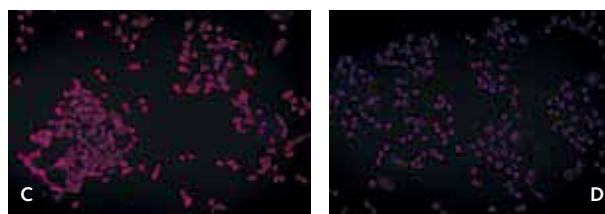
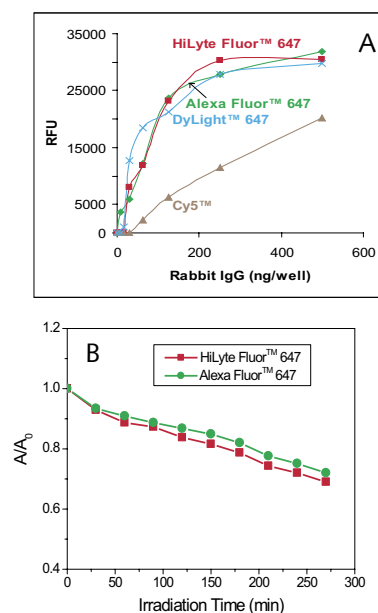


Figure 11. Performance of dye-goat anti-rabbit IgG conjugates (panel A). HiLyte Fluor™ 647 and Alexa Fluor™ 647 photostability over time (panel B). 3T3 cells incubated with anti-tubulin antibody and goat anti-rabbit antibodies, labeled either with HiLyte Fluor™ 647 (panel C), or Cy5™ (panel D), nuclei were stained with Hoechst 33342 (AS-83218).

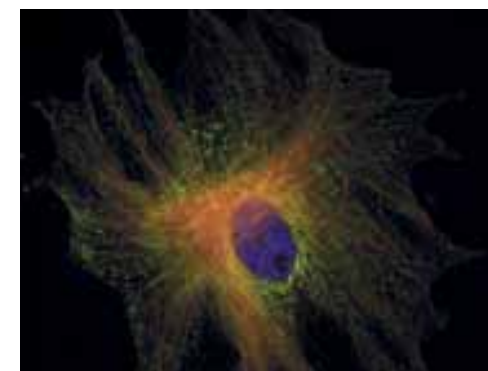


Figure 12.  $\alpha$ -Tubulins of bovine pulmonary artery endothelial cells were probed with biotin-conjugated mouse anti- $\alpha$ -tubulin, visualized with HiLyte Fluor™ 647 conjugated streptavidin (AS-60667). Mitochondria were detected with mouse anti-Oxphos V complex, visualized with HiLyte Fluor™ 488-conjugated goat anti-mouse (AS-28175-H488), nuclei stained with DAPI (AS-83210).

### HiLyte Fluor™ 647 Dye

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

## HiLyte Fluor™ 680 Dye, an excellent replacement 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 labelling. Extinction coefficient is  $190000 \text{ M}^{-1}\text{cm}^{-1}$ , with a quantum yield of 0.36.

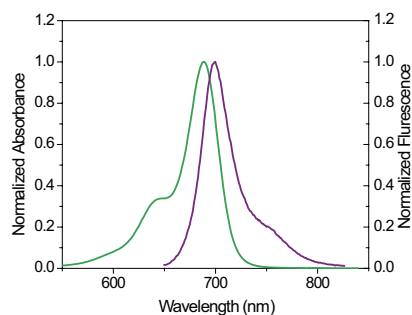


Figure 13. Absorption and emission spectra of HiLyte Fluor™ 680

### HiLyte Fluor™ 680

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

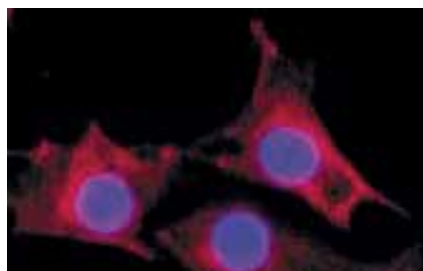


Figure 14.  $\alpha$ -Tubulin in 3T3 cells probed with mouse anti-tubulin, visualized with HiLyte Fluor™ 680-conjugated rabbit-anti-mouse IgG (AS-28164-H680), nuclei stained with DAPI (AS-83210).

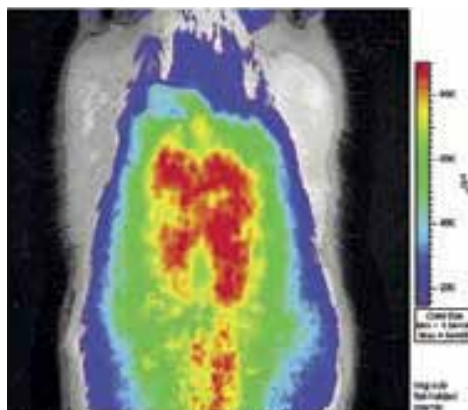


Figure 15. Image shows an in-vivo image of a rat injected with 20 nmol of c[RGDyK(HiLyte Fluor™ 750)] peptide-dye conjugate 3h post injection (image courtesy of J. Rey, Univ. of South Florida).

## HiLyte Fluor™ 750 Dye, an excellent replacement 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  $275,000 \text{ M}^{-1}\text{cm}^{-1}$ , with a quantum yield of 0.7. With a peak excitation at  $\sim 753 \text{ nm}$ , 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.

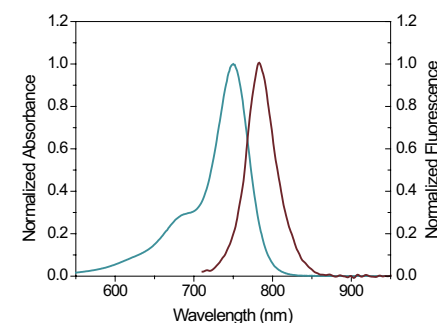


Figure 16. Absorption and emission spectra of HiLyte Fluor™ 750

### HiLyte Fluor™ 750

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

# AnaTag™ protein labelling kits

## Easy labelling with AnaTag™ protein labelling kit

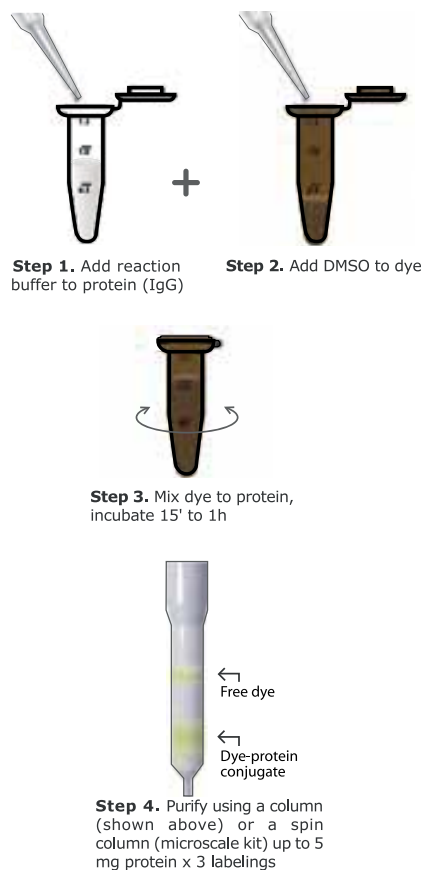


Figure 17. Labelling of an amino group (for instance, side chain of lysine) on a biopolymer with a succinimidyl ester of a dye in 4 easy steps using the AnaTag Protein Labelling Kits.

## AnaTag™ HiLyte Fluor™ protein labelling kits

The AnaTag™ HiLyte™ Protein Labelling Kits provide a convenient way to label proteins using the succinimidyl ester (SE) reactive form of the HiLyte™ 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™-protein conjugates may be used for 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 Plus™-protein conjugates.

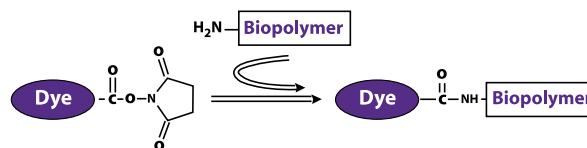


Figure 18. The succinimidyl ester (SE) group of the fluorophore reacts with the amino group of lysines on the protein to form a stable carboxamide bond.

**AnaTag™ Protein Labelling kits are available in large scale (3 x 5 mg) or microscale (3 x 200 µg) labelling sizes.**

AnaTag™ HiLyte Fluor™			
Product	Abs / Em (nm / nm)	3 x 5 mg	3 x 200 µg
AnaTag™ HiLyte Fluor™ 488 Protein Labelling Kit	499 / 523	AS-72047	AS-72048
AnaTag™ HiLyte Fluor™ 555 Protein Labelling Kit	553 / 568	AS-72045	AS-72046
AnaTag™ HiLyte Fluor™ 594 Protein Labelling Kit	596 / 617	AS-72121	AS-72120
AnaTag™ HiLyte Fluor™ TR Protein Labelling Kit	591 / 622	AS-72051 (3 x 1mg)	AS-72052
AnaTag™ HiLyte Fluor™ 647 Protein Labelling Kit	652 / 669	AS-72049	AS-72050
AnaTag™ HiLyte Fluor™ 680 Protein Labelling Kit	678 / 699	AS-72119	AS-72118
AnaTag™ HiLyte Fluor™ 750 Protein Labelling Kit	754 / 778	AS-72043	AS-72044

## Biotins, Avidins and Streptavidins

The avidin/streptavidin-biotin interaction is the strongest known non-covalent biological interaction ( $K_d=10\text{-}15M^{-1}$ ) between a protein and its ligand. The bond formation between biotin and avidin/streptavidin is very rapid and, once formed, is unaffected by pH, organic solvents and other denaturing agents. The avidin-biotin complex can even withstand 3 M guanidine. Both avidin and streptavidin have essentially irreversible biotin-binding properties since bound biotin can only be released by denaturing the subunits of the proteins. The tight and specific binding of biotin and its derivatives to various avidins has been extensively explored for a number of biological applications.

Biotin-X, SE(d-Biotin-amidocaproateN-hydroxysuccinimide ester) is amino-reactive biotinylating reagent for peptides and proteins; it has a better avidin-binding affinity than biotin. Biotin-X, SE contains a six-carbon spacer arm that reduces steric hindrance associated with binding four biotinylated molecules per one avidin and results in enhanced detection sensitivity. The succinimidyl ester (SE) reactive form of biotin-X reacts with the amine group on the protein and forms stable carboxamide bonds, which is identical to natural peptide bonds. Biotin-X-protein conjugates are very stable and can withstand the conditions of immunofluorescent staining, fluorescence *in situ* hybridization, flow cytometry and other biological applications without hydrolysis. The kit has all essential components for performing the conjugation reaction and for purifying the biotin-protein conjugates.

### Biotin Protein Labelling Kits

Product	Kit size	Reference
AnaTag™ Biotin Microscale Protein Labelling Kit	3 labelling of 200 µg protein	AS-72058
AnaTag™ Biotin Protein Labelling Kit	3 labelling of 10 mg protein	AS-72057

HiLyte Fluor™-Streptavidin conjugate has been optimised in fluorophore/protein labelling ration to ensure high fluorescent signal and uncompromised streptavidin function.

### Streptavidin conjugates

Conjugates	Abs/Emission (nm)	Reference
Fluor™ 405 labeled	404/428	AS-60672-H405
Fluor™ 488 labeled	501/527	AS-60665
Fluor™ 555 labeled	550/566	AS-60666
Fluor™ 594 labeled	593/616	AS-60672-H594
Fluor™ TR labeled	591/622	AS-60671
Fluor™ 647 labeled	649/674	AS-60667
Fluor™ 680 labeled	678/699	AS-60659-H680
Fluor™ 750 labeled	753/778	AS-60659-H750

## Phycobiliproteins and Their Conjugates

Phycobiliproteins are a family of highly soluble and fluorescent proteins derived from cyanobacteria and eukaryotic algae. In these organisms, they are used as accessory or antenna pigments for the collection of photosynthetic light. Phycobiliproteins absorb energy in parts of the visible spectrum that are poorly utilized by chlorophyll and convey the energy to chlorophyll at the photosynthetic reaction center through fluorescence resonance energy transfer.

### Phycobiliproteins

Product	Kit Size	Abs/Em (nm)	Reference
AnaTag™ APC Protein Labelling Kit	For labelling 1 mg	650 / 660	AS-72111
AnaTag™ BPE Protein Labelling Kit	For labelling 1 mg	545 (+563) / 578	AS- 72112
AnaTag™ RPE Protein Labelling Kit	For labelling 1 mg	565 (+498, +539) / 578	AS- 72113

APC : allophycocyanin, BPE : β-Phycocerythrin, RPE : R-Phycocerythrin

## Reactive fluorescent dyes

Reactive fluorescent dyes are widely used to modify amino acids, peptides, proteins (in particular, antibodies), oligonucleotides, nucleic acids, carbohydrates and other biological molecules.

Among the reactive dyes, amine-reactive dyes are most often used to prepare various bioconjugates for immunochemistry, histochemistry, fluorescence *in situ* hybridization (FISH), cell tracing, receptor binding and other biological applications since amino groups are either abundant or easily introduced into biomolecules. In general, thiol-reactive reagents are frequently used to develop probes for investigating some particular protein structures and functions. Additionally, some amine-containing fluorescent reagents are also used to modify biomolecules, in particular, to label glycoproteins. Compared to amino and thiol groups, hydroxy and carboxy groups are less frequently used to label biopolymers.

### Reactive fluorescent dyes

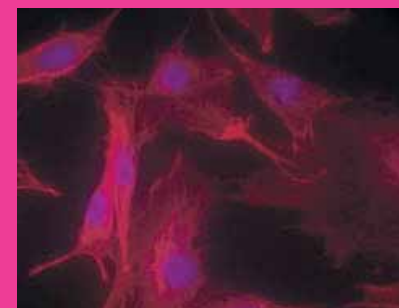
Product example	Size	Reference
5 - TAMRA, SE	5 mg	AS-81125
DEAC, SE	25 mg	AS-81211
1 - Pyrenebutanoic acid, succinimidyl ester	100 mg	AS-81238
EDANS Iodoacetamide	100 mg	AS-81431
N - (1 - Pyrene)maleimide	100 mg	AS-81436
Tetramethylrhodamine - 5 - maleimide	5 mg	AS-81446
5 - FITC cadaverine	5 mg	AS-81504
5(6) - TAMRA cadaverine	10 mg	AS-81506

### Reactive fluorescent dyes

Product	Kit size	Reference
AnaTag™ 5 - FAM Microscale Protein Labelling Kit	3 labellings of 200 µg protein	AS-72054
AnaTag™ 5 - FAM Protein Labelling Kit	3 labellings of 5 mg protein	AS-72053
AnaTag™ 5 - FITC Microscale Protein Labelling Kit	3 labellings of 200 µg protein	AS-72060
AnaTag™ 5 - FITC Protein Labelling Kit	3 labellings of 5 mg protein	AS-72059
AnaTag™ 5 - ROX Microscale Protein Labelling Kit	3 labellings of 200 µg protein	AS-72062
AnaTag™ 5 - ROX Protein Labelling Kit	3 labellings of 5 mg protein	AS-72061
AnaTag™ 5 - TAMRA Microscale Protein Labelling Kit	3 labellings of 200 µg protein	AS-72064
AnaTag™ 5 - TAMRA Protein Labelling Kit	3 labellings of 5 mg protein	AS-72063
AnaTag™ AMCA - X Microscale Protein Labelling Kit	3 labellings of 200 µg protein	AS-72056
AnaTag™ AMCA - X Protein Labelling Kit	3 labellings of 5 mg protein	AS-72055

Other fluorescent dyes & conditioning are available on [www.eurogentec.com](http://www.eurogentec.com)

Your unique requirements in terms of dyes can come to life !



Please contact our skilled chemists to colour your project  
[info@eurogentec.com](mailto:info@eurogentec.com)