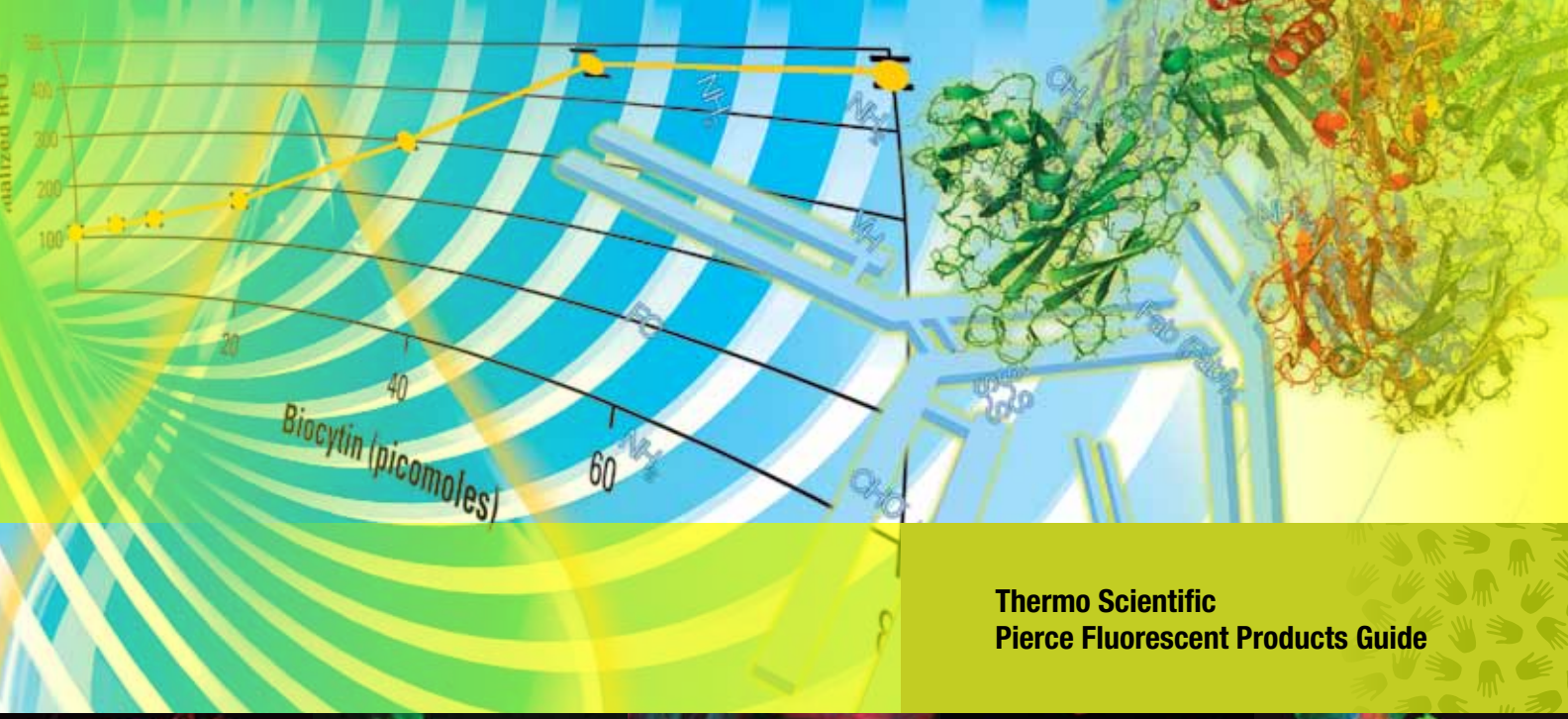
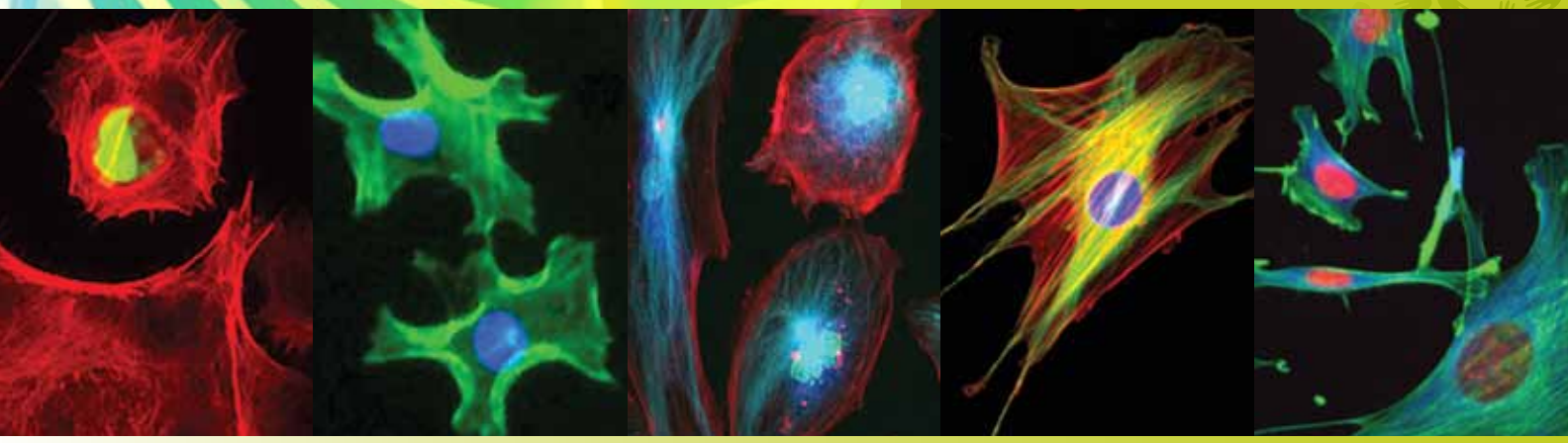


Thermo Scientific
Pierce Fluorescent Products Guide



fluorescent
labeling and detection

Thermo Scientific DyLight Dyes and Conjugates • Dye Removal Columns
Antibody Labeling Kits • MW Markers

Thermo
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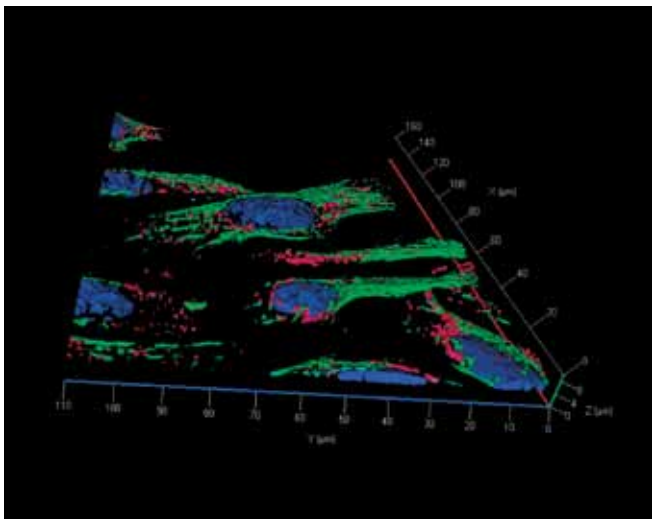


DyLight Dye
glowing advantages

Bright new alternatives to other fluorescent dyes

Thermo Scientific DyLight Amine-Reactive and Sulfhydryl-Reactive Fluorescent Dyes

DyLight® Dyes have absorption spectra ranging from 353nm to 770nm (Table 1) and match the principal output wavelengths of common fluorescence instrumentation. The DyLight Dyes exhibit higher fluorescence intensity and photostability than Alexa Fluor®, CyDye® and LI-COR® Dyes in many applications and remain highly fluorescent over a broad pH range (pH 4-9). Additionally, the water solubility of the DyLight Dyes allows a high dye-to-protein ratio without precipitation during conjugation.

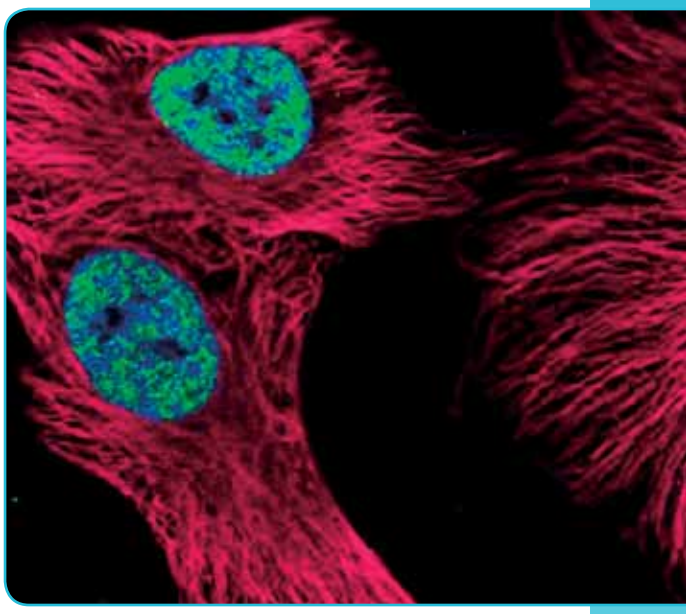


Figure 1. Thermo Scientific DyLight 488 and DyLight 633 Dyes exhibit outstanding fluorescence in structured illumination. The uniform fluorescence intensity throughout the images demonstrates the outstanding brightness and photostability of DyLight 488 and 633 Dyes. **Red:** Alpha-tubulin detected in HeLa cells with anti-tubulin monoclonal antibody and DyLight 633 Dye-conjugated secondary antibody (highly cross-adsorbed). **Green:** Histone H4 detected with anti-histone monoclonal antibody and DyLight 488 Dye-conjugated secondary antibody (highly cross-adsorbed). **Blue:** Nucleus counter-stained with fluorescent mounting media containing DAPI. Images were acquired with the Axio Imager™ Z1 and ApoTome™ Slider (Zeiss MicroImaging, Inc). The ApoTome Module provides confocal-like resolution allowing optical sectioning without using a pinhole (e.g., confocal). No image enhancement was performed.

Table 1. Spectral properties of Thermo Scientific DyLight Fluorescent Dyes.

Emission	DyLight Dye	Ex/Em*	ε†	Spectrally Similar Dyes
Blue	350	353/432	15,000	AMCA, Alexa Fluor 350 Dye
Blue	405	400/420	30,000	Alexa Fluor 405 and Cascade Blue® Dyes
Green	488	493/518	70,000	Alexa Fluor 488, fluorescein and FITC Dyes
Yellow	550	556/576	80,000	Alexa Fluor 546, Alexa Fluor 555, Cy®3 and TRITC Dyes
Red	594	593/618	80,000	Alexa Fluor 594 and Texas Red® Dyes
Red	633	638/658	170,000	Alexa Fluor 633 Dye
Red	650	652/677	250,000	Alexa Fluor 647 and Cy5 Dyes
Near-IR	680	692/712	140,000	Alexa Fluor 680 and Cy5.5 Dyes
Near-IR	755	754/776	220,000	Alexa Fluor 750 and Cy7 Dyes
Near-IR	800	777/790	270,000	IRDye® 800 Dye

*Excitation and emission maxima in nanometers (± 4nm)
† Molar extinction coefficient (M^{-1} cm^{-1})

fluorescent labeling

Excellent photostability make these dyes the clear alternative

Thermo Scientific DyLight Amine-Reactive and Sulfhydryl-Reactive Dyes

Highlights:

- Available in both amine- and sulfhydryl-reactive chemistries for fast and efficient labeling of IgG or other proteins
- High water solubility
- Excellent photostability
- Compatible with common fluorescence instrumentation

Applications:

- Fluorescence microscopy
- Western blot detection
- Protein arrays
- Flow cytometry
- ELISA
- FRET-based technology
- And many more

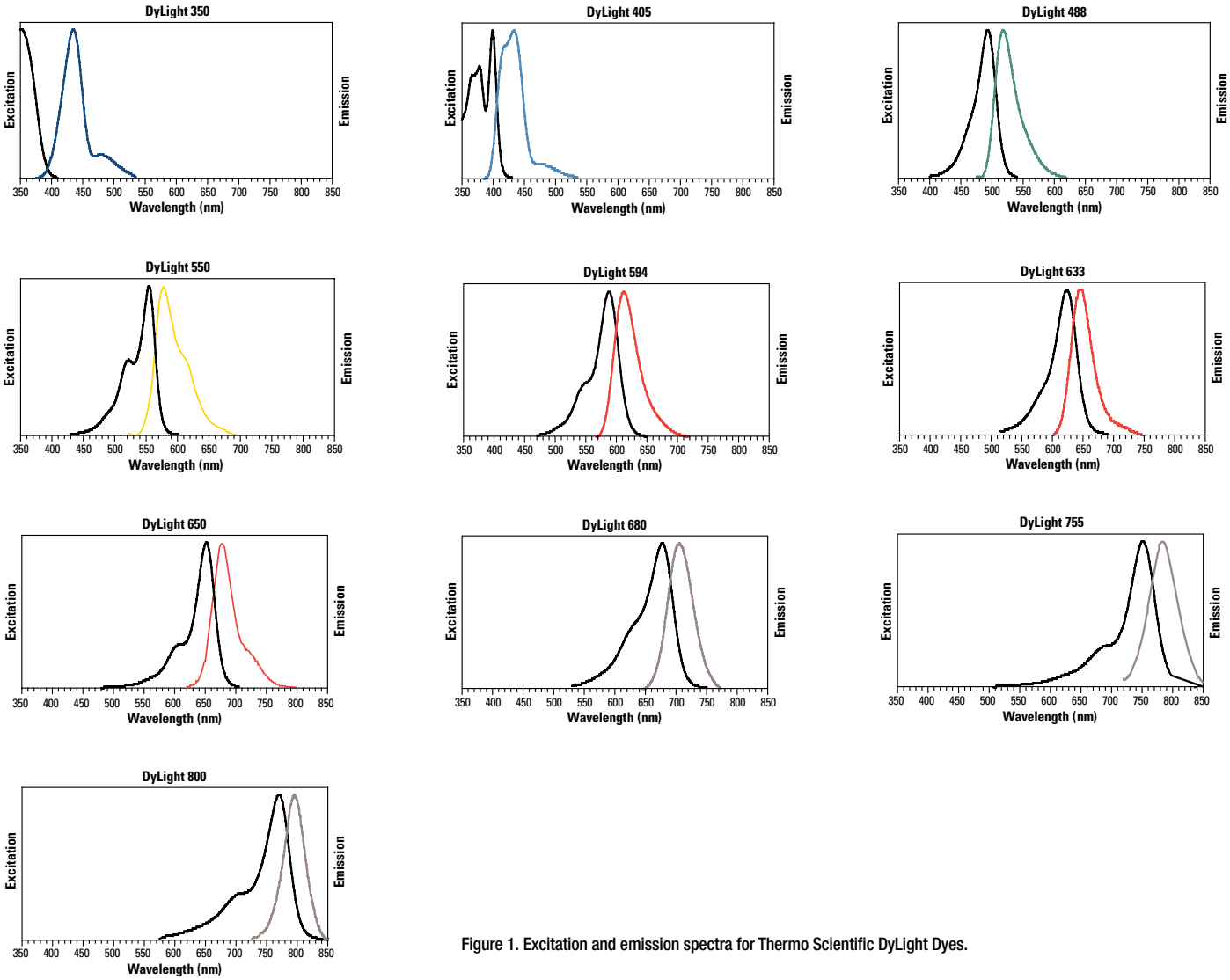


Figure 1. Excitation and emission spectra for Thermo Scientific DyLight Dyes.

Ordering Information

Product #	Description	Pkg. Size
Amine-Reactive Dyes		
46426	DyLight 350 NHS Ester	1mg
46427	DyLight 350 NHS Ester	5 x 65µg
46400	DyLight 405 NHS Ester	1mg
46401	DyLight 405 NHS Ester	5 x 50µg
46402	DyLight 488 NHS Ester	1mg
46403	DyLight 488 NHS Ester	5 x 50µg
62262	DyLight 550 NHS Ester	1mg
62263	DyLight 550 NHS Ester	5 x 50µg
46412	DyLight 594 NHS Ester	1mg
46413	DyLight 594 NHS Ester	5 x 65µg
46414	DyLight 633 NHS Ester	1mg
46417	DyLight 633 NHS Ester	5 x 50µg
62265	DyLight 650 NHS Ester	1mg
62266	DyLight 650 NHS Ester	5 x 50µg
46418	DyLight 680 NHS Ester	1mg
46419	DyLight 680 NHS Ester	5 x 50µg
62278	DyLight 755 NHS Ester	1mg
62279	DyLight 755 NHS Ester	5 x 50µg
46421	DyLight 800 NHS Ester	1mg
46422	DyLight 800 NHS Ester	5 x 50µg

Product #	Description	Pkg. Size
Sulfhydryl-Reactive Dyes		
46622	DyLight 350 Maleimide	1mg
46600	DyLight 405 Maleimide	1mg
46602	DyLight 488 Maleimide	1mg
62290	DyLight 550 Maleimide	1mg
46608	DyLight 594 Maleimide	1mg
46613	DyLight 633 Maleimide	1mg
62295	DyLight 650 Maleimide	1mg
46618	DyLight 680 Maleimide	1mg
62298	DyLight 755 Maleimide	1mg
46621	DyLight 800 Maleimide	1mg

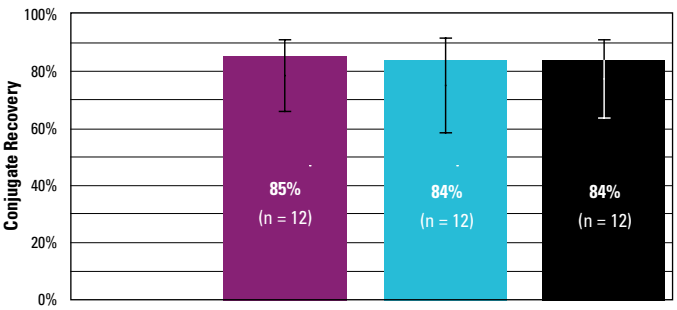
High-performance Dye Removal Columns

Convenient new options for fluorescent antibody labeling.

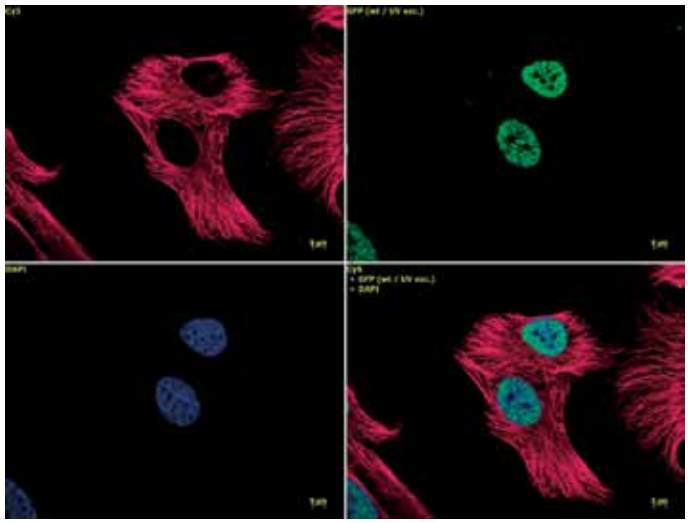
The same high-performance dye removal spin columns used in the Thermo Scientific DyLight Antibody Labeling Kits are now available separately.

Highlights:

- Fast – remove free dye from conjugates in approximately 30 seconds
- Efficient – purification resin provides outstanding conjugate recovery (Figure 1)
- Minimal sample dilution

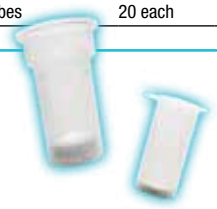


Conjugate	FITC	Fluorescein	Rhodamine
Goat anti-mouse IgG	79%	86%	94%
Streptavidin	90%	81%	74%
Average Recovery	***85%***	***84%***	***84%***



Ordering Information

Product #	Description	Pkg. Size
22858	Dye Removal Columns	Kit
	Includes: Purification Resin	5mL
	Spin Columns	10 each
	Microcentrifuge Collection Tubes	20 each



fluorescent labeling

Excellent photostability make these dyes the clear alternative

Thermo Scientific DyLight Specialty Dyes

DyLight Specialty Dyes consist of fluorophores which vary in spectral characteristics, degree of sulfonation, charge and hydrophobicity. These different characteristics allow the dyes to be used in wide range of applications, including protein coupling, imaging, FRET, microarrays and PCR.

The large selection of near infrared (NIR) and infrared (IR) dyes may be used in variety of *in vivo* and near IR fluorescence (NIRF) applications. The DyLight Specialty Dyes are offered as amine-reactive NHS esters, which can form covalent bonds with primary amines on proteins. DyLight 690-B1 and DyLight 747 near IR dyes, for use in *in vivo* applications, are also offered as non-reactive free acid dyes for use as experimental control.

Applications

Blue

- Molecular imaging
- Microscopy
- Flow cytometry

Green

- Imaging
- Antibody labeling
- Direct immunofluorescence staining
- Flow cytometry
- Fluorescence correlation spectroscopy
- ELISA
- Western blotting
- Protein microarrays
- Polymer labeling
- Peptide labeling
- Phalloidin labeling for actin staining
- Fluorescence imaging
- Fluorescence correlation spectroscopy
- Flow cytometry

Yellow

- Fluorescence imaging
- Fluorescence correlation spectroscopy
- Flow cytometry
- Imaging
- Antibody labeling
- Direct immunofluorescence staining
- ELISA
- Western blotting
- Protein microarrays
- Polymer labeling
- Peptide labeling
- Phalloidin labeling for actin staining

Orange

- Imaging
- Antibody labeling
- Direct immunofluorescence staining
- Flow cytometry
- Fluorescence correlation spectroscopy
- ELISA
- Western blotting
- Protein microarrays
- Polymer labeling
- Peptide labeling
- Phalloidin labeling for actin staining
- Staining in acidic media
- Biofilm microorganism staining

Red

- Fluorescence imaging
- Confocal microscopy
- Flow cytometry
- Spectral fluorescence imaging
- *In vivo* imaging
- Fluorescent western blotting
- Protein microarrays
- Antibody labeling
- Peptide labeling
- Fluorescence correlation spectroscopy
- Protein arrays
- Single molecule detection
- Nanoparticle conjugation
- Biotin/streptavidin conjugation

Far Red

- Fluorescence imaging
- Confocal microscopy
- Flow cytometry
- Spectral fluorescence imaging
- *In vivo* imaging
- Fluorescent western blotting
- Protein microarrays
- Antibody labeling
- Peptide labeling
- Fluorescence correlation spectroscopy
- Protein arrays
- Single molecule detection
- Nanoparticle conjugation
- Biotin/streptavidin conjugation

Near IR

- *In vivo* or *ex vivo* imaging
- Tumor imaging with labeled peptides
- NIR fluorescence (NIRF) imaging of labeled silica nanoparticles
- NIR *in vitro* imaging and characterization
- Determination of thermal stability
- Cytotoxicity assays
- Molecular imaging
- UV-VIS-NIR spectroscopy
- Fluorescence correlation spectroscopy
- MRI applications
- DNA sequencing
- Primer labeling for PCR
- 2-D gel electrophoresis
- Flow cytometry/fluorescence-activated cell sorting (FACS)
- Laser scanning confocal microscopy

Ordering Information

Product #	Fluorescent Dye	Emission Color	Ex/Em*	ε†	Base Structure	Pkg. Size
46609	DyLight 415-Co1 NHS Ester	Blue	418/463	34,000	Coumarin, 1 Sulfonate	1mg
46611	DyLight 530-R2 NHS Ester	Green	533/554	100,000	Rhodamine, 2 Sulfonates	1mg
46612	DyLight 554-R0 NHS Ester	Yellow	553/581	100,000	Rhodamine, 0 Sulfonate	1mg
46614	DyLight 554-R1 NHS Ester	Yellow	548/574	100,000	Rhodamine, 1 Sulfonate	1mg
46617	DyLight 590-R2 NHS Ester	Orange	581/598	120,000	Rhodamine, 2 Sulfonates	1mg
46624	DyLight 610-B1 NHS Ester	Orange	610/632	80,000	Benzopyrillium, 1 Sulfonate	1mg
46625	DyLight 615-B1 NHS Ester	Red	623/643	200,000	Benzopyrillium, 1 Sulfonate	1mg
46631	DyLight 633-B1 NHS Ester	Red	638/658	200,000	Benzopyrillium, 1 Sulfonate	1mg
46632	DyLight 633-B2 NHS Ester	Red	637/657	200,000	Benzopyrillium, 2 Sulfonates	1mg
46633	DyLight 633-B3 NHS Ester	Red	636/658	200,000	Benzopyrillium, 3 Sulfonates	1mg
46634	DyLight 635-B2 NHS Ester	Red	636/658	200,000	Benzopyrillium, 2 Sulfonates	1mg
46635	DyLight 655-B1 NHS Ester	Far Red	656/676	220,000	Benzopyrillium, 1 Sulfonate	1mg
46636	DyLight 655-B2 NHS Ester	Far Red	656/676	220,000	Benzopyrillium, 2 Sulfonates	1mg
46637	DyLight 655-B3 NHS Ester	Far Red	653/676	220,000	Benzopyrillium, 3 Sulfonates	1mg
46638	DyLight 655-B4 NHS Ester	Far Red	654/675	220,000	Benzopyrillium, 4 Sulfonates	1mg
46646	DyLight 675-B1 NHS Ester	Near Infrared	675/699	180,000	Benzopyrillium, 1 Sulfonate	1mg
46647	DyLight 675-B2 NHS Ester	Near Infrared	675/699	180,000	Benzopyrillium, 2 Sulfonates	1mg
46648	DyLight 675-B3 NHS Ester	Near Infrared	674/698	180,000	Benzopyrillium, 3 Sulfonates	1mg
46649	DyLight 675-B4 NHS Ester	Near Infrared	673/694	180,000	Benzopyrillium, 4 Sulfonates	1mg
53003	DyLight 679-C5 NHS Ester	Near Infrared	679/698	200,000	Cyanine, 5 Sulfonates	1mg
53022	DyLight 690-B1 NHS Ester	Near Infrared	691/709	140,000	Benzopyrillium, 1 Sulfonate	1mg
53023	DyLight 690-B1 Free Acid	Near Infrared	691/709	140,000	Benzopyrillium, 1 Sulfonate	1mg
53026	DyLight 690-B2 NHS Ester	Near Infrared	692/709	140,000	Benzopyrillium, 2 Sulfonates	1mg
53028	DyLight 700-B1 NHS Ester	Near Infrared	707/728	140,000	Benzopyrillium, 1 Sulfonate	1mg
53030	DyLight 700-B2 NHS Ester	Near Infrared	709/730	140,000	Benzopyrillium, 2 Sulfonates	1mg
53032	DyLight 730-B1 NHS Ester	Near Infrared	734/755	240,000	Benzopyrillium, 1 Sulfonate	1mg
53033	DyLight 730-B2 NHS Ester	Near Infrared	736/755	240,000	Benzopyrillium, 2 Sulfonates	1mg
53036	DyLight 730-B3 NHS Ester	Near Infrared	736/755	240,000	Benzopyrillium, 3 Sulfonates	1mg
53037	DyLight 730-B4 NHS Ester	Near Infrared	733/755	240,000	Benzopyrillium, 4 Sulfonates	1mg
62283	DyLight 747-B1 Free Acid	Near Infrared	751/774	270,000	Benzopyrillium, 1 Sulfonate	1mg
53040	DyLight 747-B2 NHS Ester	Near Infrared	751/774	270,000	Benzopyrillium, 2 Sulfonates	1mg
53041	DyLight 747-B3 NHS Ester	Near Infrared	750/771	270,000	Benzopyrillium, 3 Sulfonates	1mg
53042	DyLight 747-B4 NHS Ester	Near Infrared	748/775	270,000	Benzopyrillium, 4 Sulfonates	1mg
53054	DyLight 775-B2 NHS Ester	Infrared	772/787	240,000	Benzopyrillium, 2 Sulfonates	1mg
53055	DyLight 775-B3 NHS Ester	Infrared	770/788	240,000	Benzopyrillium, 3 Sulfonates	1mg
85150	DyLight 775-B4 NHS Ester	Infrared	767/787	240,000	Benzopyrillium, 4 Sulfonates	1mg
53064	DyLight 780-B1 NHS Ester	Infrared	783/799	170,000	Benzopyrillium, 1 Sulfonate	1mg
53065	DyLight 780-B2 NHS Ester	Infrared	784/796	170,000	Benzopyrillium, 2 Sulfonates	1mg
53066	DyLight 780-B3 NHS Ester	Infrared	785/794	170,000	Benzopyrillium, 3 Sulfonates	1mg
53067	DyLight 830-B2 NHS Ester	Infrared	844/875	220,000	Benzopyrillium, 2 Sulfonates	1mg

** Excitation and emission maxima in nanometers (± 4 nm)*
† Molar extinction coefficient (M^{-1} cm^{-1})

Thermo Scientific DyLight Long Stoke Shift (LS) Dyes

Thermo Scientific DyLight LS Dyes are fluorescent dyes with long stokes shifts (between 80-140nm) compared to traditional cyanine-based dyes in which the excitation and emission wavelengths are separated by approximately 20nm. DyLight LS Dyes absorption maximum are approximately 480nm. Their emission wavelengths depend on the substituent pattern and range from 550-680nm.

The emission wavelengths of the DyLight LS Dyes allow for multicolor detection using a single excitation source and are designed to match other widely used dyes. These dyes may be used in a variety of applications including conjugations to streptavidin, antibodies, nucleotides, oligonucleotides and silica particles for use in microscopy, FISH, flow cytometry, FRET, DNA sequencing and real-time PCR applications. These long stokes shift dyes have advantages for multiplex applications as well as photostability and quantum yield.

Applications

- Quenchers and FRET pairs
- Conjugation to silica particles
- STED microscopy
- Multicolor microscopy
- 4Pi microscopy
- Dual-color 3-D nanoscopy
- Dynamic light scattering
- Graded stokes shift
- Duplex enhanced RT-PCR
- High-resolution optical microscopy

Ordering Information

Product #	Fluorescent Dye	Emission Color	Ex/Em*	ε†	Base Structure	Pkg. Size
82492	DyLight 485-LS NHS Ester	Yellow	485/559	50,000	Coumarin	1mg
82493	DyLight 510-LS NHS Ester	Orange	509/590	50,000	Coumarin	1mg
82491	DyLight 515-LS NHS Ester	Red	519/648	50,000	Coumarin	1mg
82495	DyLight 521-LS NHS Ester	Far Red	526/666	50,000	Coumarin	1mg

** Excitation and emission maxima in nanometers (± 4 nm)*
† Molar extinction coefficient (M^{-1} cm^{-1})

Thermo Scientific DyLight Fluorescent Quenchers

DyLight Fluorescent Quenchers are a family of amine-reactive, non-fluorescent dyes. They can be efficiently paired with many DyLight Dyes and other common fluorescent dyes ranging from fluorescein, rhodamine, Texas Red, cyanine to near infrared for use in fluorescence resonance energy (FRET) assay.

Applications

- FRET
- Other quenching applications

Ordering Information

Product #	Fluorescent Dye	Absorption	ε†	Base Structure	Pkg. Size
84512	DyLight 425Q NHS Ester	425	25,000	Azo	1mg
84513	DyLight 504Q NHS Ester	504	39,000	Azo	1mg
84508	DyLight 543Q NHS Ester	544	44,000	Azo	1mg
84509	DyLight 641Q NHS Ester	641	105,000	Benzopyrillium	1mg
84514	DyLight 662Q NHS Ester	661	140,000	Benzopyrillium	1mg
84510	DyLight 683Q NHS Ester	680	80,000	Acridinium	1mg
84515	DyLight 696Q NHS Ester	696	49,000	Azo	1mg
84511	DyLight 766Q NHS Ester	766	180,000	Benzopyrillium	1mg

** Excitation and emission maxima in nanometers (± 4 nm)*
† Molar extinction coefficient (M^{-1} cm^{-1})

Thermo Scientific DyLight Antibody Labeling Kits

These kits were specifically developed for fast, efficient labeling of antibodies. Two convenient kit formats are available to accommodate varied labeling requirements. The Antibody Labeling Kits contain all necessary components to perform three separate labeling reactions using 1mg of IgG or similar quantities of other proteins. The DyLight Microscale Antibody Labeling Kits contain all the necessary components to perform five separate labeling reactions using 100µg of IgG. The labeling kits use high-performance spin desalting columns to provide exceptional dye removal and antibody recovery (Figure 1).

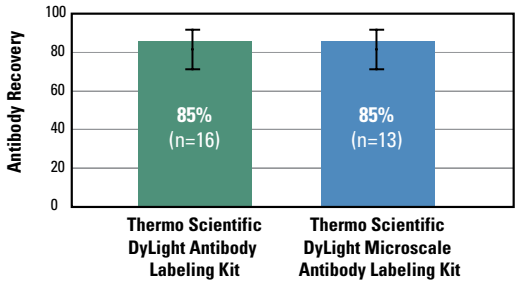


Figure 1. Thermo Scientific DyLight Antibody Labeling Kits provide outstanding recovery. The percent recovery for DyLight Antibody Labeling Kits is the average for 16 labeling reactions using three different antibodies. The percent recovery for DyLight Microscale Antibody Labeling Kits is the average for 13 labeling reactions using three different antibodies.

Highlights:

- Fast – fluorescently label and purify protein in approximately one hour
- Amine-reactive dyes – label virtually any protein
- Pre-measured fluorescent dye – eliminate the time, waste and hassle associated with weighing dye
- Efficient non-reacted dye removal
- Minimal sample dilution
- Spin column format eliminates the need for column preparation, fraction screening and waiting for protein to emerge from column
- Easy protocol (Figure 2)

Table 1. Spectral properties of Thermo Scientific DyLight Fluorescent Dyes.

Emission	DyLight Dye	Ex/Em*	ε†	Spectrally Similar Dyes
Blue	350	353/432	15,000	AMCA, Alexa Fluor 350 Dye
Blue	405	400/420	30,000	Alexa Fluor 405 and Cascade Blue Dyes
Green	488	493/518	70,000	Alexa Fluor 488, fluorescein and FITC Dyes
Yellow	550	556/576	150,000	Alexa Fluor 546, Alexa Fluor 555, Cy3 and TRITC Dyes
Red	594	593/618	80,000	Alexa Fluor 594 and Texas Red® Dyes
Red	633	638/658	170,000	Alexa Fluor 633 Dye
Red	650	652/677	250,000	Alexa Fluor 647 and Cy5 Dyes
Near-IR	680	692/712	140,000	Alexa Fluor 680 and Cy5.5 Dyes
Near-IR	755	754/776	220,000	Alexa Fluor 750 and Cy7 Dyes
Near-IR	800	777/790	270,000	IRDye® 800 Dye

*Excitation and emission maxima in nanometers (± 4 nm)
† Molar extinction coefficient (M^{-1} cm^{-1})

Microscale Kits

Contains sufficient reagent to label and purify 5 x100µg of IgG.

All Microscale Kits include:

- Appropriate DyLight NHS Ester, 5 vials
- Reaction Buffer, 1mL
- Spin Columns, 5 each
- Microcentrifuge Collection Tubes, 10 each
- Purification Resin, 5mL

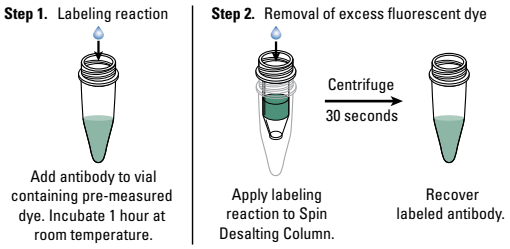


Figure 2. Protocol summary for Thermo Scientific DyLight Antibody Labeling Kits.

Ordering Information

Product #	Description	Pkg. Size
62276	DyLight 350 Microscale Antibody Labeling Kit DyLight 350 NHS Ester	Kit 5 vials
53021	DyLight 405 Microscale Antibody Labeling Kit DyLight 405 NHS Ester	Kit 5 vials
53025	DyLight 488 Microscale Antibody Labeling Kit DyLight 488 NHS Ester	Kit 5 vials
84531	DyLight 550 Microscale Antibody Labeling Kit DyLight 550 NHS Ester	Kit 5 vials
53045	DyLight 594 Microscale Antibody Labeling Kit DyLight 594 NHS Ester	Kit 5 vials
53047	DyLight 633 Microscale Antibody Labeling Kit DyLight 633 NHS Ester	Kit 5 vials
84536	DyLight 650 Microscale Antibody Labeling Kit DyLight 650 NHS Ester	Kit 5 vials
53057	DyLight 680 Microscale Antibody Labeling Kit DyLight 680 NHS Ester	Kit 5 vials
84539	DyLight 755 Microscale Antibody Labeling Kit DyLight 755 NHS Ester	Kit 5 vials
53063	DyLight 800 Microscale Antibody Labeling Kit DyLight 800 NHS Ester	Kit 5 vials

Antibody Labeling Kits

Contains sufficient reagent to label and purify 3 x 1mg of IgG or similar quantities of other proteins.

All Antibody Labeling Kits include:

- Appropriate DyLight NHS Ester, 3 vials
- Reaction Buffer, 1mL
- Spin Columns, 6 each
- Microcentrifuge Collection Tubes, 12 each
- Purification Resin, 5mL

Ordering Information

Product #	Description	Pkg. Size
62275	DyLight 350 Antibody Labeling Kit DyLight 350 NHS Ester	Kit 3 vials
53020	DyLight 405 Antibody Labeling Kit DyLight 405 NHS Ester	Kit 3 vials
53024	DyLight 488 Antibody Labeling Kit DyLight 488 NHS Ester	Kit 3 vials
84530	DyLight 550 Antibody Labeling Kit DyLight 550 NHS Ester	Kit 3 vials
53044	DyLight 594 Antibody Labeling Kit DyLight 594 NHS Ester	Kit 3 vials
53046	DyLight 633 Antibody Labeling Kit DyLight 633 NHS Ester	Kit 3 vials
84535	DyLight 650 Antibody Labeling Kit DyLight 650 NHS Ester	Kit 3 vials
53056	DyLight 680 Antibody Labeling Kit DyLight 680 NHS Ester	Kit 3 vials
84538	DyLight 755 Antibody Labeling Kit DyLight 755 NHS Ester	Kit 3 vials
53062	DyLight 800 Antibody Labeling Kit DyLight 800 NHS Ester	Kit 3 vials

Thermo Scientific DyLight Fluor Phosphine Reagents

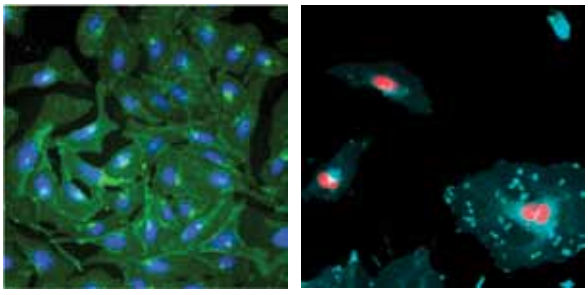
DyLight Fluor Phosphine Reagents are phosphine-activated fluorescent dyes for specific labeling and detection of azide-tagged molecules, which enables use of fluorescence imaging in metabolic labeling strategies.

Azide groups, which are the reactive targets of the phosphine-activated fluors, can be introduced into proteins or other cellular targets via azide reagents or through *in vivo* labeling with azide-derivatives of naturally occurring metabolic building blocks. Because neither phosphines nor azides are present in biological systems, they comprise chemoselective (mutually specific) ligation pairs for labeling and conjugation.

Thus, when used in combination with azide labeling strategies, Phosphine-Activated DyLight Fluors enable directed fluorescent labeling to facilitate detection of protein interactions and post-translational modifications using fluorescence imaging technologies.

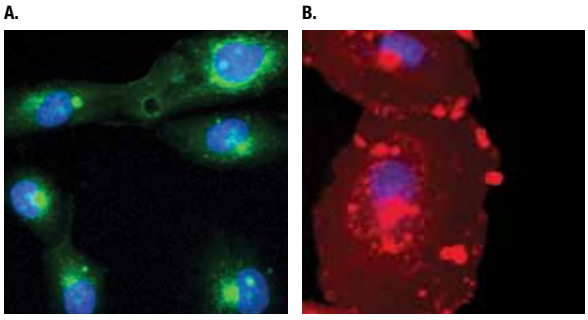
Highlights:

- **Soluble** – easily dissolves in water-miscible solvents (e.g., DMSO) for subsequent dilution in aqueous reaction mixtures with cell lysates and other biological samples
- **Compatible** – reaction chemistry occurs effectively in simple buffer conditions; requires no accessory reagents such as copper or reducing agents, and does not interfere with fluorescence applications
- **Chemoselective** – the phosphine reactive group is specific in biological samples for azide-tagged molecules, ensuring that fluorescent labeling is specific
- **High-performance fluorescence** – DyLight 488, DyLight 550 and DyLight 650 Dyes are intense, highly stable fluorophores for green, orange and red fluorescent detection.



U2OS: azido-galactosamine **HK-2: azido-mannosamine**

***In vivo* detection of metabolically incorporated azido-acetylgalactosamine using Thermo Scientific DyLight 550- and 650-Phosphine Labeling Reagents. Panel A.** U2OS cells were incubated with 40µM azido-acetylgalactosamine in cell culture media for 72 hours and the live cells were incubated with 100µM of DyLight 550-phosphine . The cells were then washed, fixed with 4% paraformaldehyde and counterstained with Hoechst 33342. (**green:** DyLight 550-labeled azido-galactosamine, **blue:** Hoechst 33342 labeled nuclei). **Panel B.** HK-2 cells were incubated with 40µM azido-acetylmannosamine in cell culture media for 72 hours and the live cells were incubated with 100µM of DyLight 650-phosphine. The cells were then washed, fixed with 4% paraformaldehyde and counterstained with Hoechst 33342. (**cyan:** DyLight 650-labeled azido-mannosamine, **red:** Hoechst 33342 labeled nuclei).



HK-2: fixed-cell labeling **HK-2: live-cell labeling**

Detection of metabolically incorporated azido-sugars on live and fixed cells using Thermo Scientific DyLight DyLight 550- and 650-Phosphine Labeling Reagents. HK-2 cells were incubated with 40µM azido-acetylmannosamine in cell culture media for 72 hours. The azido-sugar were labeled either after 4% paraformaldehyde fixation using DyLight 550-phosphine (Panel A) or labeled in live cells with DyLight 650-Phosphine Labeling Reagent (Panel B). The cells were washed and counterstained with Hoechst 33342. Panel A. The golgi structure (green) is predominantly detected by fixed cell labeling, where as the cell membrane and secretory vesicles (red) are labeled by live-cell labeling (blue: Hoechst 33342 labeled nuclei).

Ordering Information

Product #	Description	Pkg. Size
88907	DyLight 488-Phosphine Sufficient for 1 use to label a total of 6mg of IgG using typical conditions	1mg
88910	DyLight 550-Phosphine Sufficient for 1 use to label a total of 5mg of IgG using typical conditions	1mg
88911	DyLight 650-Phosphine Sufficient for 1 use to label a total of 5mg of IgG using typical conditions	1mg

Thermo Scientific Fluorescein Dyes

Fluorescein dyes are commonly used for fluorescent detection. We offer both amine-reactive and sulfhydryl-reactive versions of fluorescein.

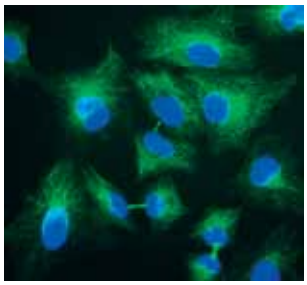
NHS-fluorescein and fluorescein isothiocyanate (FITC), two reactive derivatives of fluorescein dye, are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA.

FITC is the base fluorescein molecule functionalized with an isothiocyanate reactive group (-N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive toward primary amine groups on proteins, peptides and other biomolecules. A succinimidyl-ester functional group attached to the fluorescein core, creating NHS-fluorescein, forms another common derivative that has much greater specificity toward primary amines in the presence of other nucleophiles and a more stable linkage following labeling.

Our Fluorescein is a mixture of isomers with reactive groups attached at the five and six positions of the bottom ring (See Structure). The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.

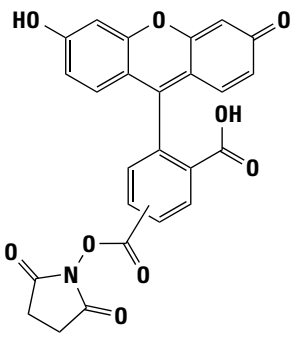
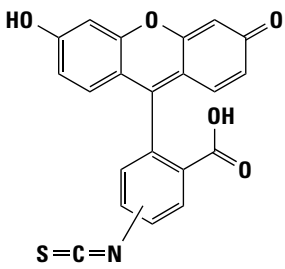
Fluorescein-5-maleimide and 5-Iodoacetamidofluorescein (5-IAF) are sulfhydryl-reactive derivatives of fluorescein dye. Fluorescein-5-maleimide is the base fluorescein molecule functionalized with a maleimide reactive group by replacing a hydrogen atom on the bottom ring of the structure. 5-IAF is the core fluorescein molecule functionalized with an iodoacetamide group. Both fluorescein derivatives are reactive toward sulfhydryl groups (e.g., reduced cysteine residues) on proteins, peptides and other biomolecules.

A derivative of fluorescein, DyLight 488 Fluor, has been tailored for various chemical and biological applications where greater photostability and fluorescence intensity, pH independence, and a narrower emission spectrum are required.

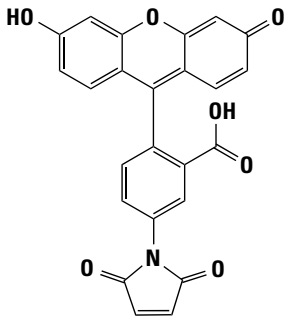
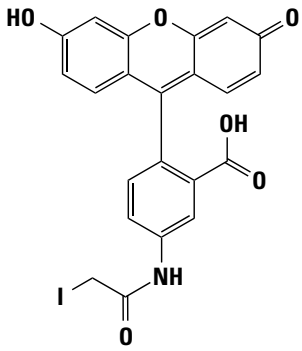


The Thermo Scientific NHS-Fluorescein Antibody Labeling Kit (Product # 53029) produces ideal conjugates for immunofluorescence. A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps® X-100 (Product # 28314). The cells were then probed with a 0.4µg/ml mouse anti-α-tubulin antibody and 2µg/ml fluorescein-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse TS100 fluorescent microscope using Zeiss AxioCam™ camera and AxioVison™ software.

Properties of amine-reactive fluorescein dyes.

	NHS-Fluorescein	FITC
Structure	 **NHS-Fluorescein MW 473.39**	 **FITC MW 389.38**
Alternative names	5/6-FAM SE	5/6-FITC
Chemical name	5/6-carboxyfluorescein succinimidyl ester	5(6)-fluorescein isothiocyanate mixed isomer
Molecular weight	473.4	389.2
Excitation source	488nm spectral line, argon-ion laser	488nm spectral line, argon-ion laser
Excitation wavelength	494nm	494nm
Emission wavelength	518nm	518nm
Extinction coefficient	> 70,000/M^{-1} cm^{-1}	> 70,000/M^{-1} cm^{-1}
CAS #	117548-22-8	27072-45-3
Purity	> 90% by HPLC	> 95% by HPLC
Solubility	Soluble in DMF or DMSO	Soluble in aqueous buffers at pH > 6
Storage	Desiccated at -20°C, protect from moisture, use only fresh solutions	Desiccated at -20°C, protect from moisture, use only fresh solutions
Reactive groups	NHS ester, reacts with primary amines at pH 7.0 to 9.0	Isothiocyanate, reacts with primary amines at pH 7.0 to 9.0 Other

Properties of sulfhydryl-reactive dyes.

	Fluorescein-5-maleimide	5-Iodoacetamido-fluorescein
Structure	 **Fluorescein-5-Maleimide MW 427.36**	 **5-IAF 5-Iodoacetamido-fluorescein MW 515.25**
Alternative names	5-MF, 5-maleimido-fluorescein	5-IAF, 5-iodoacetamidofluorescein
Chemical name	1H-Pyrrole-2,5-dione, 1-(3',6'-dihydroxy-3-oxospiro(isobenzofuran-1(3H),9'-(9H)xanthen-5-yl)-	Acetamide, N-(3',6'-dihydroxy-3-oxospiro(isobenzofuran-1(3H), 9'-(9H) xanthen)-5-yl)-2-iodo
Molecular weight	427.36 ±3	515.26 ±3
Excitation source	488nm spectral line, argon-ion laser	488nm spectral line, argon-ion laser
Excitation wavelength	494nm	494nm
Emission wavelength	518nm	518nm
Extinction coefficient	~ 68,000/M^{-1} cm^{-1}	> 80,000/M^{-1} cm^{-1}
CAS #	75350-46-8	63368-54-7
Solubility	Soluble in DMF or DMSO	Soluble in DMF; aqueous buffers at pH > 6
Storage	Desiccated at -20°C, protect from moisture, use only fresh solutions	Desiccated at -20°C, protect from moisture, use only fresh solutions
Reactive groups	Maleimide, reacts with sulfhydryls at pH 6.5 to 7.5	Iodoacetamide, reacts with sulfhydryls at pH 7.0 to 7.5

Ordering Information

Product #	Description	Pkg. Size
46424	**FITC (Fluorescein Isothiocyanate)**	1g
46425	**FITC (Fluorescein Isothiocyanate)**	100mg
46409	**NHS-Fluorescein**	1g
46410	**NHS-Fluorescein**	100mg
53027	**FITC Antibody Labeling Kit** Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour. Includes: FITC Borate Buffer Spin Columns Microcentrifuge Collection Tubes Purification Resin	Kit 3 vials 1mL 6 each 12 each 5mL

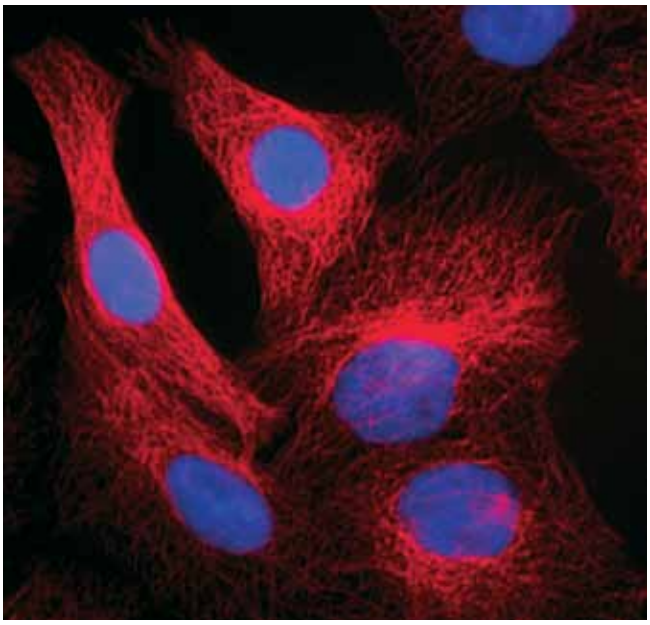
Product #	Description	Pkg. Size
53029	**Fluorescein Antibody Labeling Kit** Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour. Includes: NHS Fluorescein Borate Buffer Spin Columns Microcentrifuge Collection Tubes Purification Resin	Kit 3 vials 1mL 6 each 12 each 5mL
62245	**Fluorescein-5-Maleimide**	25mg
62246	**5-Iodoacetamido-fluorescein (5-IAF)**	25mg

Thermo Scientific Rhodamine Dyes

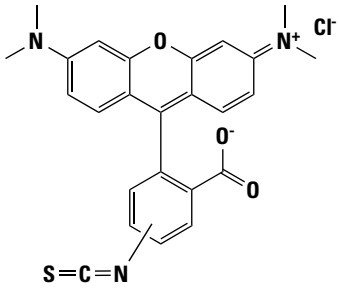
NHS-rhodamine and tetramethylrhodamine isothiocyanate TRITC, two reactive derivatives of rhodamine dye, are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA.

TRITC is the base tetramethylrhodamine molecule functionalized with an isothiocyanate reactive group (-N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive toward amine and sulfhydryl groups on proteins, peptides and other biomolecules. A succinimidyl-ester functional group attached to the tetramethylrhodamine core, creating NHS-fluorescein, forms another common derivative that has much greater specificity toward primary amines in the presence of other nucleophiles and a more stable linkage following labeling. Texas Red Sulfonyl Chloride is a long-wavelength derivative of rhodamine that is modified with sulfonyl chloride for reaction to primary amines.

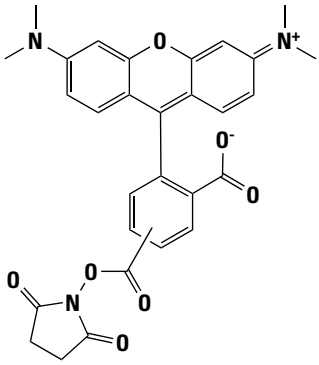
Our Rhodamine Dyes are a mixture of isomers with reactive groups attached at the five and six positions of the bottom ring (See Structure). The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.



The Thermo Scientific NHS-Rhodamine Antibody Labeling Kit (Product # 53031) produces ideal conjugates for immunofluorescence. A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps X-100 (Product # 28314). The cells were then probed with a 0.4µg/mL mouse anti-α-tubulin antibody and 2µg/mL rhodamine-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse TS100 fluorescent microscope using Zeiss AxioCam camera and AxioVision software.



TRITC
MW 478.97
Em/Ex 544/572



NHS-Rhodamine
MW 527.52
Em/Ex 552/575

Ordering Information

Product #	Description	Pkg. Size
46112	**TRITC (Tetramethylrhodamine Isothiocyanate)**	10mg
46406	**NHS-Rhodamine**	25mg
53031	**Rhodamine Antibody Labeling Kit** Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.	Kit
	Includes: NHS Rhodamine	3 vials
	Borate Buffer	1mL
	Spin Columns	6 each
	Microcentrifuge Collection Tubes	12 each
	Purification Resin	5mL

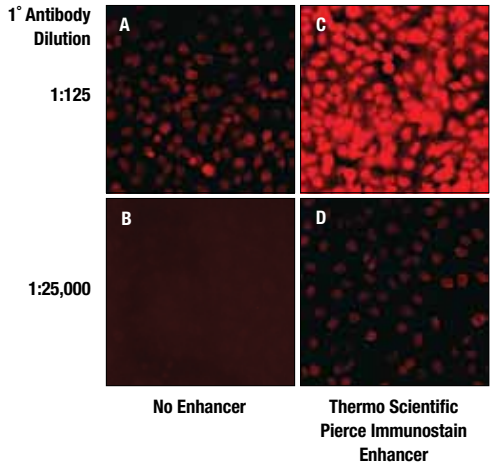
Alleviates common immunostaining problems such as low signal and poor sensitivity

Thermo Scientific Pierce Immunostain Enhancer

The Pierce Immunostain Enhancer is compatible with fluorescent and chromogenic detection and routinely increases both signal intensity and detection sensitivity. Signal enhancement is antibody-dependent and typically ranges from 3- to 12-fold. Because of the strong signal enhancement, the Pierce Immunostain Enhancer reduces the amount of antibody required to achieve optimal detection.

Highlights:

- **Save precious antibody** – Pierce Immunostain Enhancer allows the customer to use only a fraction of antibody to achieve the same signal as with conventional immunodetection
- **Convenience** – simply replace your current antibody dilution buffer with Pierce Immunostain Enhancer (unlike other signal enhancement methods which require additional steps)
- **Increased signal intensity and sensitivity** – provides 3- to 12-fold increase in signal intensity and sensitivity for improved visualization of the antigen of interest in cells and tissues
- **Improved specificity** – significantly improves signal-to-noise ratio for poor quality and low affinity antibodies
- **Compatible** – can be used with chromogenic and fluorescent detection methods



Save money by using only a fraction of your precious primary antibody by diluting in Thermo Scientific Pierce Immunostain Enhancer (200-fold dilution of Lamin B1 primary antibody beyond the vendor's recommendation). A549 cells were seeded at 5000 cells per well in a 96-well plate and incubated for 18-20 hours at 37°C, 5% CO_2 in a humidified incubator. The cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Thermo Scientific Surfact-Amps X-100 and then blocked at room temperature (RT) for 30 minutes with 2% BSA Blocker containing 0.1% Triton® X-100 (standard buffer). The primary and the secondary antibodies were diluted with either the standard buffer or with Thermo Scientific Pierce Immunostain Enhancer. A549 cells were probed with rabbit polyclonal anti-lamin B1 for 1 hour at room temperature, and then the lamin B1 was detected with Thermo Scientific DyLight 594-conjugated goat anti-rabbit antibody (4µg/mL) (Product # 35560). Lamin B1 antibody specifically stains the nuclear envelope. **Panel A:** Lamin B1 is detected in A549 cells using a 1:125 dilution of the primary antibody in BSA; **Panel B:** The same dilution results in oversaturation of the signal when the Pierce Immunostain Enhancer is used. **Panel C:** The antigen is undetectable when the primary antibody is diluted 1:25,000 in standard buffer; **Panel D:** Pierce Immunostain Enhancer results in clear detection of lamin B1 at the same dilution as Panel C. Images were acquired at the same gain and exposure time using appropriate optical filter sets with a 20X (0.45 NA) objective.

Ordering Information

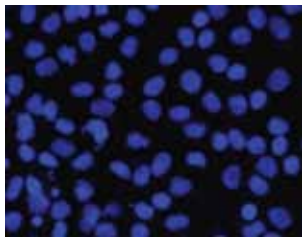
Product #	Description	Pkg. Size
46644	Pierce Immunostain Enhancer Sufficient for 100 large (~3cm²) tissue section slides.	20mL
46645	Pierce Immunostain Enhancer Sufficient for 10 large (~3cm²) tissue section slides.	2mL

Thermo Scientific DAPI Stain

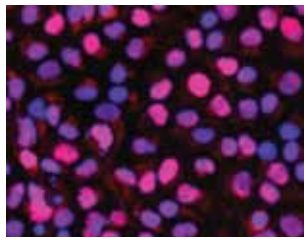
DAPI reagents are high-purity forms of DAPI dye for fixed-cell, fluorescent staining of DNA content and nuclei for cellular imaging techniques.

DAPI (diamidino-2-phenylindole) is a blue fluorescent probe that fluoresces brightly when it is selectively bound to the minor groove of double-stranded DNA where its fluorescence is approximately 20-fold greater than in the nonbound state. This selectivity for DNA, along with cell permeability allows staining of nuclei with little background from the cytoplasm, making DAPI the classic nuclear counterstain for immunofluorescence microscopy. DAPI has greater photostability than Hoechst dyes, another common nuclear counterstain, when it is bound to double-stranded DNA. DAPI has an excitation maximum at 345nm and an emission maximum at 455nm. DAPI is compatible with fluorescein and rhodamine dyes, as well as with DyLight and Alexa Fluor Dye, for nuclear counterstaining of DNA in fluorescence imaging.

As a counterstain in fluorescence imaging methods, DAPI is compatible with antibodies and other probes labeled with fluorescein and rhodamine dyes, as well as with DyLight Fluors. DAPI has greater photostability than Hoechst dyes, although Hoechst 33342 can be use for live cell imaging while use of DAPI is confined to fixed cells. DAPI is offered in powdered solid and aqueous solution forms.



DAPI

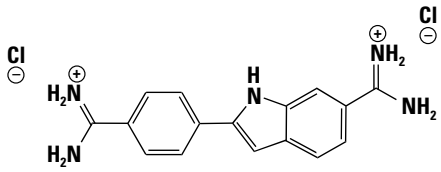


DAPI + HMGB

Dual imaging with DAPI and antibody probes. DU145 prostate cancer cells were grown in 96-well plates, fixed with paraformaldehyde and permeabilized for 15 minutes. Cells were incubated for 1 hour at room temperature with a mouse anti-human HMGB antibody, then for 45 minutes with Thermo Scientific DyLight 549 Goat Anti-Mouse Secondary Antibody. Finally, Thermo Scientific DAPI was added at 1µg/mL for 5 minutes. Stained cells were imaged with the Thermo Scientific ArrayScan VTI Instrument (Obj. 20X/0.45NA).

Properties of Fluorescent Dye.

Structure



DAPI
4,6'-Diamidino-2-phenylindole dihydrochloride
MW 350.25

Alternative names	DAPI Stain, DAPI Dye, DNA Content Counterstain
Chemical name(s)	4,6'-diamidino-2-phenylindole, dihydrochloride 4',6-diamidine-2-phenyl indole 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride
Molecular formula	$C_{16}H_{15}N_5$ 2HCl
Molecular weight	350.25
Excitation wavelength	341 ±3nm (near 360nm when bound to dsDNA)
Emission wavelength	452 ±3nm (456 to 460nm when bound to dsDNA)
Extinction coefficient	> 30,600/M^{-1} cm^{-1} at 347nm in methanol
CAS #	28718-90-3
Purity	> 95% (most lots >98%) by HPLC at 240nm
Solubility	> 1mg/mL in water; compound is soluble in DMF, water and various non-phosphate aqueous buffers
Storage	Store solid at room temperature (RT), protected from light Store DAPI solution (1mg/mL) at -20°C protected from light
Reactive groups	None; binds to minor groove of double-stranded DNA

Ordering Information

Product #	Description	Pkg. Size
62247	**DAPI** Formulation: 4',6-Diamidino-2-phenylindole, dihydrochloride; powder.	10mg
62248	**DAPI Solution** Formulation: 4',6-Diamidino-2-phenylindole, dihydrochloride; 1mg/mL aqueous solution.	1mL

Thermo Scientific Hoechst 33342 Stain

Hoechst 33342 Solution is a high-quality form of Hoechst dye for fixed- and live-cell fluorescent staining of DNA and nuclei in cellular imaging techniques.

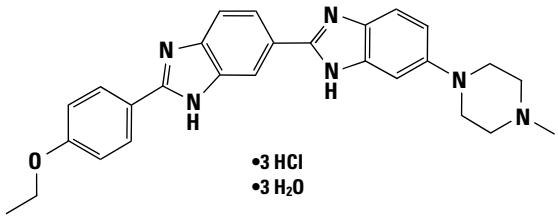
Hoechst 33342 (2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole trihydrochloride trihydrate) is a cell-permeable DNA stain that is excited by ultraviolet light and emits blue fluorescence at 460-490nm. Hoechst 33342 binds preferentially to adenine-thymine (A-T) regions of DNA. This stain binds into the minor groove of DNA and exhibits distinct fluorescence emission spectra that are dependent on dye:base pair ratios.

Hoechst 33342 is used for specifically staining the nuclei of living or fixed cells and tissues. This stain is commonly used in combination with 5-bromo-2'-deoxyuridine (BrdU) labeling to distinguish the compact chromatin of apoptotic nuclei, to identify replicating cells and to sort cells based on their DNA content. A combination of Hoechst 33342 and propidium iodide have been extensively used for simultaneous flow cytometric and fluorescence imaging analysis of the stages of apoptosis and cell-cycle distribution.

As a counterstain in fluorescent imaging, Hoechst dye is compatible with antibodies and other probes labeled with fluorescein and rhodamine dyes, as well as with Thermo Scientific DyLight Fluors. The stable 20mM aqueous stock solution is essentially ready for use.

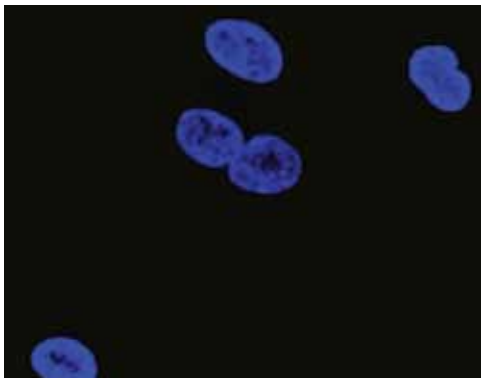
Properties of Hoescht 33342 Dye.

Structure



Hoechst 33342
MW 615.99

Alternative names	Hoechst Stain, Hoechst Dye, DNA Content Counterstain
Chemical name	2'-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl) -2,5'-bi-1H-benzimidazole trihydrochloride trihydrate
Molecular formula	$C_{27}H_{28}N_6O$ •3HCl •$3H_2O$
Molecular weight	615.99
Excitation wavelength	346 ±3nm (361nm when bound to dsDNA)
Emission wavelength	497 ±3nm
Extinction coefficient	Source compound ~47,000/M^{-1} cm^{-1} (> 45,000) at 343nm in methanol
CAS #	28491-52-3
Purity	> 95% (most lots >98%) by HPLC at 240nm
Solubility	Product is supplied at 20mM (12.3mg/mL) in water; Hoechst dye is soluble in DMF, water and various non-phosphate aqueous buffers
Storage	Store supplied solution at 2 to 8°C protected from light
Reactive groups	None; dye binds to minor groove of double-stranded DNA



Hoechst 33342

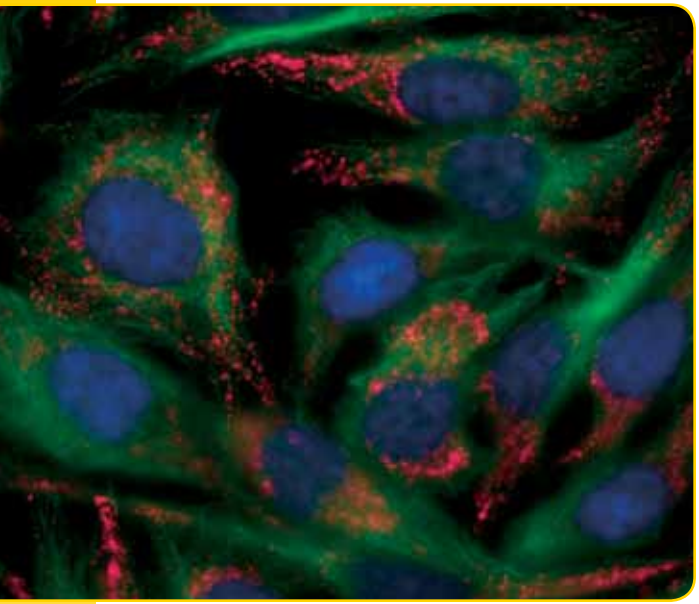
Cellular imaging with Hoechst 33342. A549 human lung cancer cells were grown on 96-well plates, fixed with paraformaldehyde and permeabilized for 15 minutes. Thermo Scientific Hoechst 33342 Solution was added at 1µg/mL for 5 minutes. Stained cells were imaged with the ArrayScan® VTI Instrument (Obj. 20X/0.45NA).

Ordering Information

Product #	Description	Pkg. Size
62249	**Hoechst 33342 Solution** Formulation: 12.3mg/mL (20mM) aqueous solution.	5mL

DyLight conjugates

Excellent brightness make these conjugates a clear alternative



Thermo Scientific DyLight Conjugates

Pre-conjugated secondary antibodies and biotin-binding proteins of DyLight Fluors make excellent secondary detection reagents for fluorescent assays. Our DyLight Fluor Conjugates are optimally labeled with the highest dye-to-protein ratio (F/P ratio) and does not cause quenching of the fluorescent signal or problems with conjugate solubility.

Highlights:

- Available conjugated to commonly used secondary antibodies, Streptavidin and NeutrAvidin Biotin-Binding Protein
- Molar ratio (dye:protein) optimized to provide excellent fluorescent intensity
- Stable for 1 year at 4°C
- Antibody conjugates are affinity-purified to minimize cross-reactivity

Western Blotting

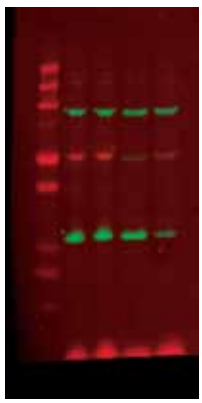


Figure 1. Two-color infrared Western blot detection of p53 and cyclophilin B knockdown using Thermo Scientific DyLight 680- and Thermo Scientific DyLight 800-labeled secondary antibodies. Protein lysate from transfected A549 cells was separated using SDS-PAGE and transferred to PVDF membrane. **Lane 1:** MW marker, **Lane 2:** mock transfected sample, **Lane 3:** negative control siRNA, **Lane 4:** siRNA targeted against p53 and **Lane 5:** siRNA targeted against cyclophilin. The membranes were imaged with the Odyssey Infrared Imaging System using the 700 and 800 channels.

Ordering Information

Conjugates: Package size for these items is 1mg at 1mg/mL.

	Product #				
Description	**DyLight 350 Dye**	**DyLight 405 Dye**	**DyLight 488 Dye**	**DyLight 550 Dye**	**DyLight 594 Dye**
Goat Anti-Mouse IgG (H+L)	62271		35502	84540	
Goat Anti-Mouse IgG Highly Cross-Adsorbed	62273	35500	35503		35511
Goat Anti-Rabbit IgG (H+L)	62270		35552	84541	
Goat Anti-Rabbit IgG Highly Cross-Adsorbed	62272	35550	35553		35561
Streptavidin			21832	84542	
NeutrAvidin Biotin-Binding Protein			22832	84606	

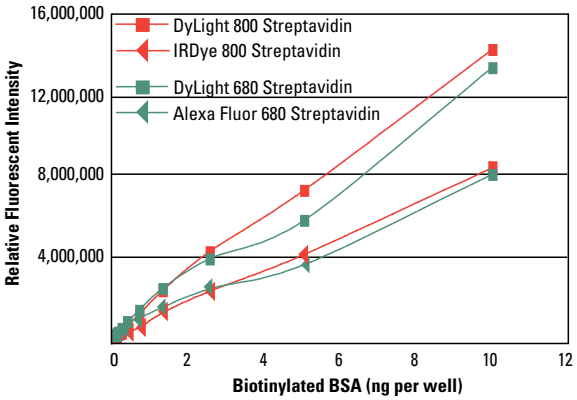


Figure 2. Thermo Scientific DyLight 680 and Thermo Scientific DyLight 800 Streptavidin Conjugates are brighter than Alexa Fluor 680 or IRDye 800 Conjugates in microplate-based assays. Microplates were coated with biotinylated BSA at the indicated concentrations. Conjugates were diluted to 10µg/mL in PBS and 100µL was applied to each plate. Fluorescent intensity was measured with the Odyssey Infrared Imaging System using the 700 and 800 channels.

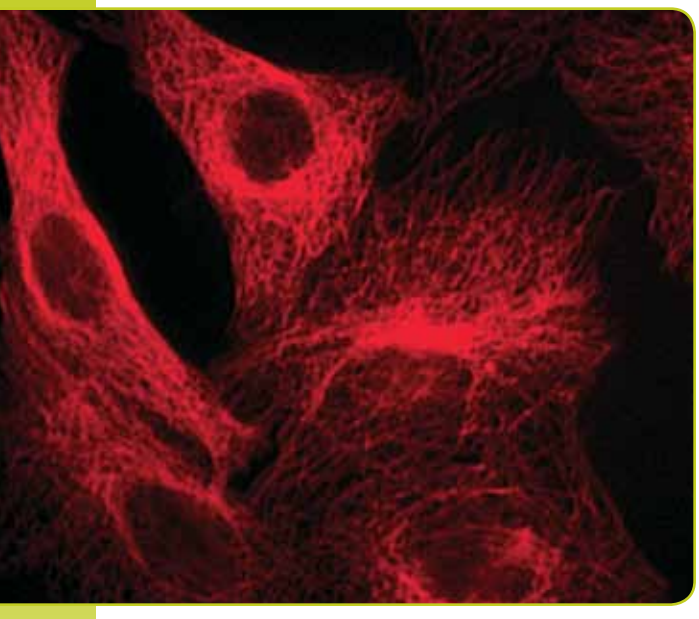
Ordering Information

Conjugates: Package size for these items is 1mg at 1mg/mL.

	Product #			
Description	**DyLight 633 Dye**	**DyLight 650 Dye**	**DyLight 680 Dye**	**DyLight 800 Dye**
Goat Anti-Mouse IgG (H+L)		84545	35518	35521
Goat Anti-Mouse IgG Highly Cross-Adsorbed	35513		35519	
Goat Anti-Rabbit IgG (H+L)		84546	35568	35571
Goat Anti-Rabbit IgG Highly Cross-Adsorbed	35563		35569	
Streptavidin		84547	21848	21851
NeutrAvidin Biotin-Binding Protein		84607	22848	22853

Near-IR dyes
for high-sensitivity applications

Infrared dyes with the LI-COR Odyssey instrument for easy, sensitive detection



Thermo Scientific DyLight 680 and 800 Near Infrared Dyes for use with the LI-COR Odyssey Instrument

Near-IR dyes are becoming more widely used in a variety of cellular assays and imaging applications. The dyes fluoresce in the 670-1,000nm range, at which biomolecules exhibit low background fluorescence. Combined with a high extinction coefficient, this low autofluorescence property allows high-sensitivity immunoassays, blotting procedures and *in vivo* imaging applications. In immunoblotting, for example, detection sensitivity with near-IR dye-conjugated antibodies are comparable with direct radioactive detection or indirect detection using enzyme-based assays. Near-IR dyes allow for a wider dynamic detection range and more reliable quantitation.

The LI-COR Odyssey Infrared Imaging System is useful for detecting infrared dyes. It is a two-channel laser-based infrared detection system made by LI-COR Biosciences. It provides high sensitivity, multiplexing capabilities and accurate quantitation. We offer a variety of products based on near-IR dyes that are compatible with the LI-COR Odyssey Imager (Figure 1), allowing the full utilization of this instrument's features.

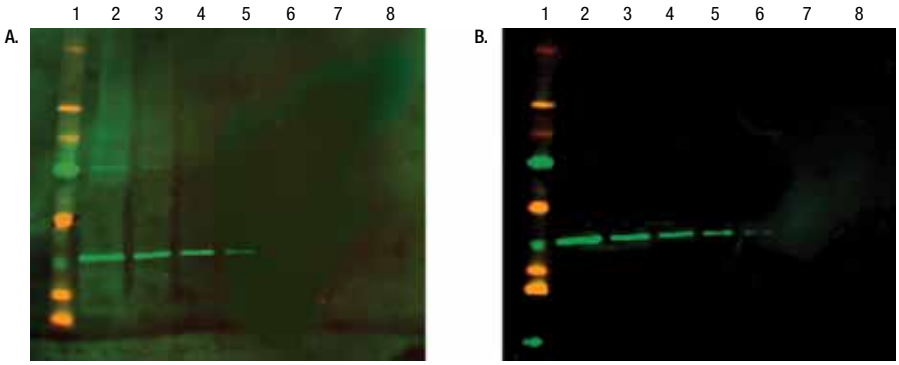


Figure 1. Detection of cyclophilin B in Hela cell lysate with rabbit anti-cyclophilin B and Thermo Scientific DyLight 800 Goat Anti-Rabbit or IRDye 800 Goat Anti-Rabbit. HeLa cell lysate was prepared under reducing conditions and loaded onto the well of two 4-20% Tris-Glycine gels. Following electrophoresis, the proteins were transferred onto low-fluorescence PVDF membrane (Product # 22860) using a semi-dry transfer unit (45 minutes at 15V). After transfer, the blots were blocked for 1 hour at room temperature in Thermo Scientific SEA BLOCK Blocking Buffer (Product # 37527). Primary antibody Rabbit Anti-Cyclophilin B (AbCam) was prepared at 0.5µg/mL in SEA BLOCK Blocking Buffer. Blots were incubated for 1 hour at room temperature, then washed 3 x 10 minutes in PBS with 0.05% Tween®-20 and incubated with the secondary labeled antibody (DyLight 800 Goat Anti-Rabbit Antibody, **Panel A** or IRDye 800 Goat Anti-Rabbit Antibody, **Panel B**) diluted in PBS with 0.05% Tween-20 at a concentration of 0.05µg/mL, each. After a one-hour incubation at room temperature, the blots were washed 6 x 5 minutes in PBS with 0.05% Tween-20 and then scanned on a LI-COR Odyssey IR Imager.

Infrared dye-based products currently offered:

- **Amine- and sulfhydryl-reactive dyes** comparable to IRDye 800 and Alexa Fluor 680 Dye
- **Labeling kits for fluorescently labeling** various amounts of primary or secondary antibodies
- **Streptavidin and NeutrAvidin® Protein-conjugated DyLight 680 and DyLight 800 Dyes**
- **Goat Anti-Mouse- and Goat Anti-Rabbit-conjugated DyLight 680 and DyLight 800 Dyes**
- **Thermo Scientific Krypton Infrared Protein Stain** for total protein detection in polyacrylamide gels – the only infrared total protein stain available (see page 24)

DyLight IR Fluors offer many benefits for labeling and imaging applications. The dyes are extremely photostable compared to other available dyes that excite and emit at the same wavelengths. In addition, DyLight Dyes exhibit high solubility, making them extremely useful for protein labeling. We offer a choice of two fluors, DyLight 680 and 800 Dyes, that are compatible with the LI-COR Odyssey System (Figure 2). We also offer the Krypton Infrared Protein Stain, which is the only infrared total protein stain available. It offers low nanogram level sensitivity and multiplexing capabilities when used with the LI-COR Odyssey.

Low-autofluorescence properties combined with high extinction coefficients allow DyLight Infrared Dyes to be used in a variety of applications, including:

- Western blotting
- Immunoassays
- In-cell Western assays
- In-gel detection
- *In vivo* imaging

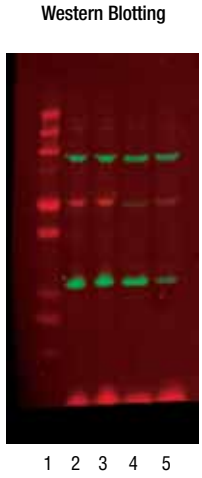


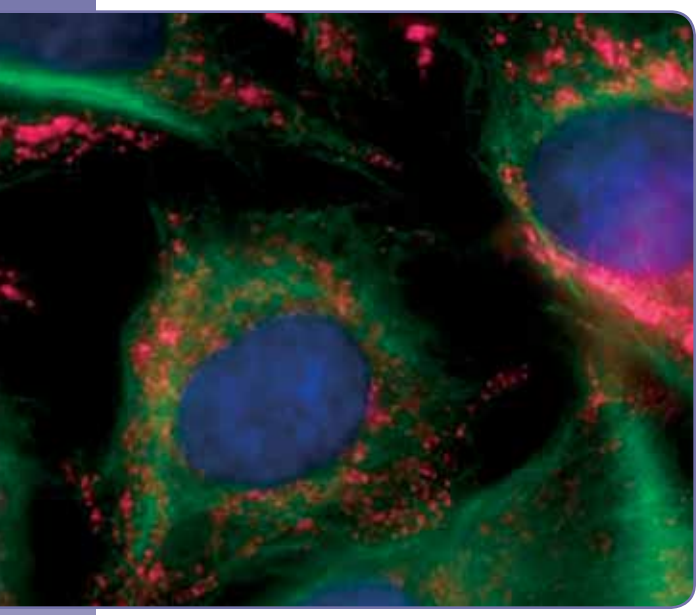
Figure 2. Two-color infrared Western blot detection of p53 and cyclophilin B knockdown using Thermo Scientific DyLight 680- and Thermo Scientific DyLight 800-labeled Secondary Antibodies. Protein lysate from transfected A549 cells was separated using SDS-PAGE and transferred to PVDF membrane. **Lane 1:** MW marker, **Lane 2:** mock transfected sample, **Lane 3:** negative control siRNA, **Lane 4:** siRNA targeted against p53 and **Lane 5:** siRNA targeted against cyclophilin. The membranes were imaged with the Odyssey Infrared Imaging System using the 700 and 800 channels.

Reference:

1. Abaitua, F. and O'Hare, P. (2008). Identification of a highly conserved, functional nuclear localization signal within the *N*-terminal region of HSV-1 VP1-2 tegument protein. *J. Virol.* **10(1128)**, 2497-07.

fluorescent quantitation

Detect down to 750ng of biotinylated IgG



Thermo Scientific Fluorescence Biotin Quantitation Kit

Accurately measure biotinylation level with a new highly sensitive fluorescent assay.

The highly specific interaction of avidin with biotin (i.e., vitamin H) is useful for designing nonradioactive purification and detection systems. Quantifying the degree of biotinylation is necessary for determining if biotinylation was successful and the amount of biotinylated molecule to use for a specific application. The Thermo Scientific Fluorescence Biotin Quantitation Kit contains a premix of fluorescent avidin with HABA and a biocytin standard.This fluorescent method is highly sensitive and accurate and requires only 10µL of sample, whereas the traditional colorimetric method requires 100µL of sample and Supplier I's fluorescent assay requires 50µL of sample. Additionally, our kit more accurately quantitates biotin than Supplier I's fluorescent assay (Figure 1).

Highlights:

- **Fast** – single requires only 5 minutes of incubation
- **Economical** – requires only 10µL of valuable samples
- **Sensitive** – linear working range of 10-60 picomoles of biotin

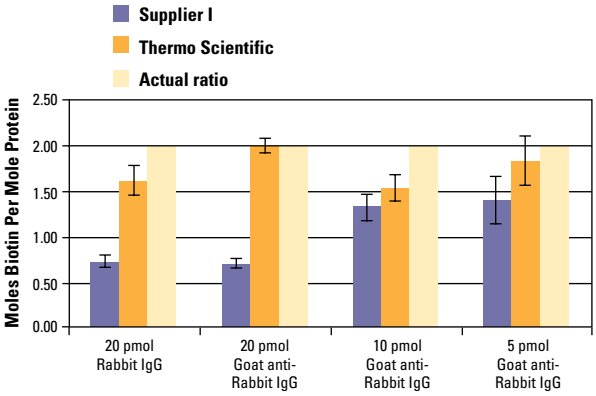


Figure 1. Comparison of results obtained using Thermo Scientific Fluorescence Biotin Quantitation Kit to those generated using a similar kit from Supplier I. Rabbit and goat anti-rabbit IgG samples were spiked with a known amount of Sulfo-NHS-LC-Biotin (Product # 21327), incubated at room temperature for 4 hours and placed at 4°C overnight. Kit protocols were performed according to the manufacturer's instructions. The standard curve for the Supplier I assay was prepared by 1:2 dilution of 1.6µM biocytin. Fifty microliters of each standard and sample were pipetted into a microplate, in triplicate, and 50µL of detection reagent was added. The mixture was incubated for 5 minutes at room temperature and assayed for fluorescence using excitation 485nm and emission 530nm. The assay was performed as described in Figure 2. A linear regression was fit to the linear portion of each standard curve and the equation used to calculate picomoles of biotin per sample. This value was divided by the picomoles of protein in the sample to generate the ratio of moles of biotin per mole of protein.

HABA (4´-hydroxyazobenzene-2-carboxylic acid) is a dye that weakly interacts with avidin and is commonly used in a colorimetric assay to quickly estimate the biotin-to-protein ratio; however, our new fluorescent method is more sensitive and accurate. The premix fluorescent avidin with HABA (Thermo Scientific DyLight Reporter) is added to the solution containing the biotinylated sample. Because of its higher affinity for avidin, biotin displaces the HABA, allowing the avidin to fluoresce. The amount of biotin is measured in a microplate by comparing the fluorescence to a biocytin standard curve (Figure 2).

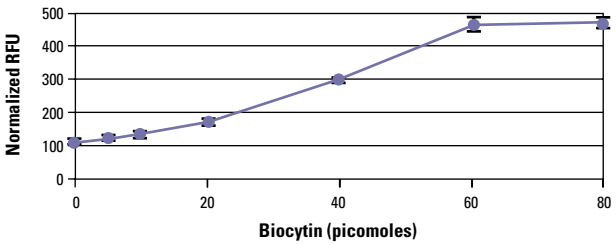


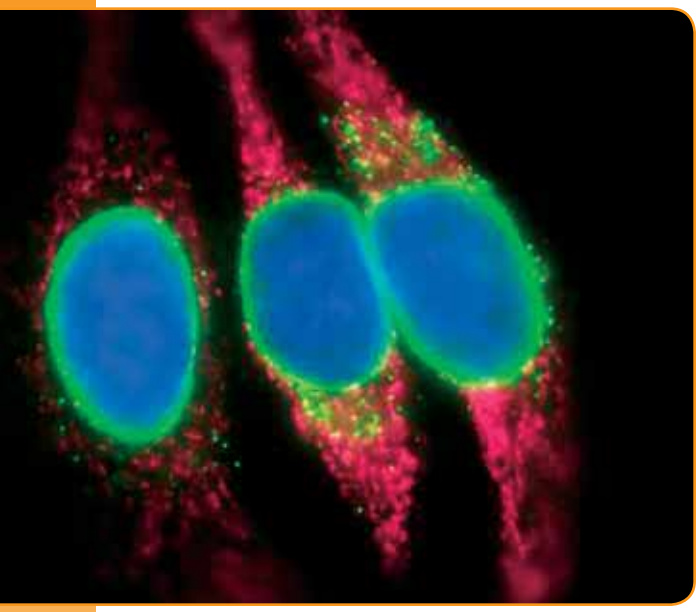
Figure 2. Normalized biocytin standard curve for the Thermo Scientific Fluorescence Biotin Quantitation Kit. The standard curve was prepared using standards of 100, 80, 60, 40, 20, 10, and 5 picomoles biocytin/10µL. Ten microliters of each standard was pipetted into a microplate, in triplicate, and 90µL of detection reagent was added. The mixtures were incubated for 5 minutes at room temperature and assayed for fluorescence using excitation 494nm and emission 520nm.

Ordering Information

Product #	Description	Pkg. Size
46610	**Fluorescence Biotin Quantitation Kit**	Kit
	Sufficient reagent to perform 200 microplate assays.	
	Contains: Phosphate Buffered Saline 20X	3mL
	DyLight Reporter	5 vials
	Biocytin Control	200µM, 100µL

fluorescent protein gel stains

A faster, affordable fluorescent stain that provides excellent performance



Thermo Scientific Krypton Fluorescent Protein Stain

Thermo Scientific Krypton Protein Stain is a fluorescent stain for detecting proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2-D gels.

Highlights:

- Excitation/emission maxima – 520/580nm
- Compatibility – works with all SDS-polyacrylamide and 2-D gel types and with MS analysis
- Linear quantitative range – three to four orders of magnitude
- Sensitive – detects down to 0.25ng protein with the basic 2.3-hour protocol
- Fast – using the rapid protocol, detects down to 2ng protein in 30 minutes
- Comparative – minimal differential staining of proteins

Ordering Information

Product #	Description	Pkg. Size
46628	Krypton Protein Stain (10X) Sufficient reagent to stain four mini gels (8 cm x 10 cm)	20mL
46629	Krypton Protein Stain (10X) Sufficient reagent to stain 20 mini gels (8 cm x 10 cm) or two to four large-format gels	100mL
46630	Krypton Protein Stain (10X) Sufficient reagent to stain 100 mini gels (8 cm x 10 cm) or 10 to 20 large-format gels	500mL

Thermo Scientific Pierce Antibody Immunostaining Guide

Immunofluorescence (IF) and immunohistochemistry (IHC) are two methods commonly used to detect proteins in a cellular context. This guide contains technical information, dyes, stains and antibodies to help you in your research of many cellular pathways, structures, organelles and processes.

Thermo Scientific Pierce Antibody Production and Purification Technical Handbook

The updated Antibody Production and Purification Technical Handbook is an essential resource for any laboratory working with antibodies. The handbook provides an overview of antibody structure and types, as well as technical information on the procedures, reagents and tools used to produce, purify, fragment and label antibodies.

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