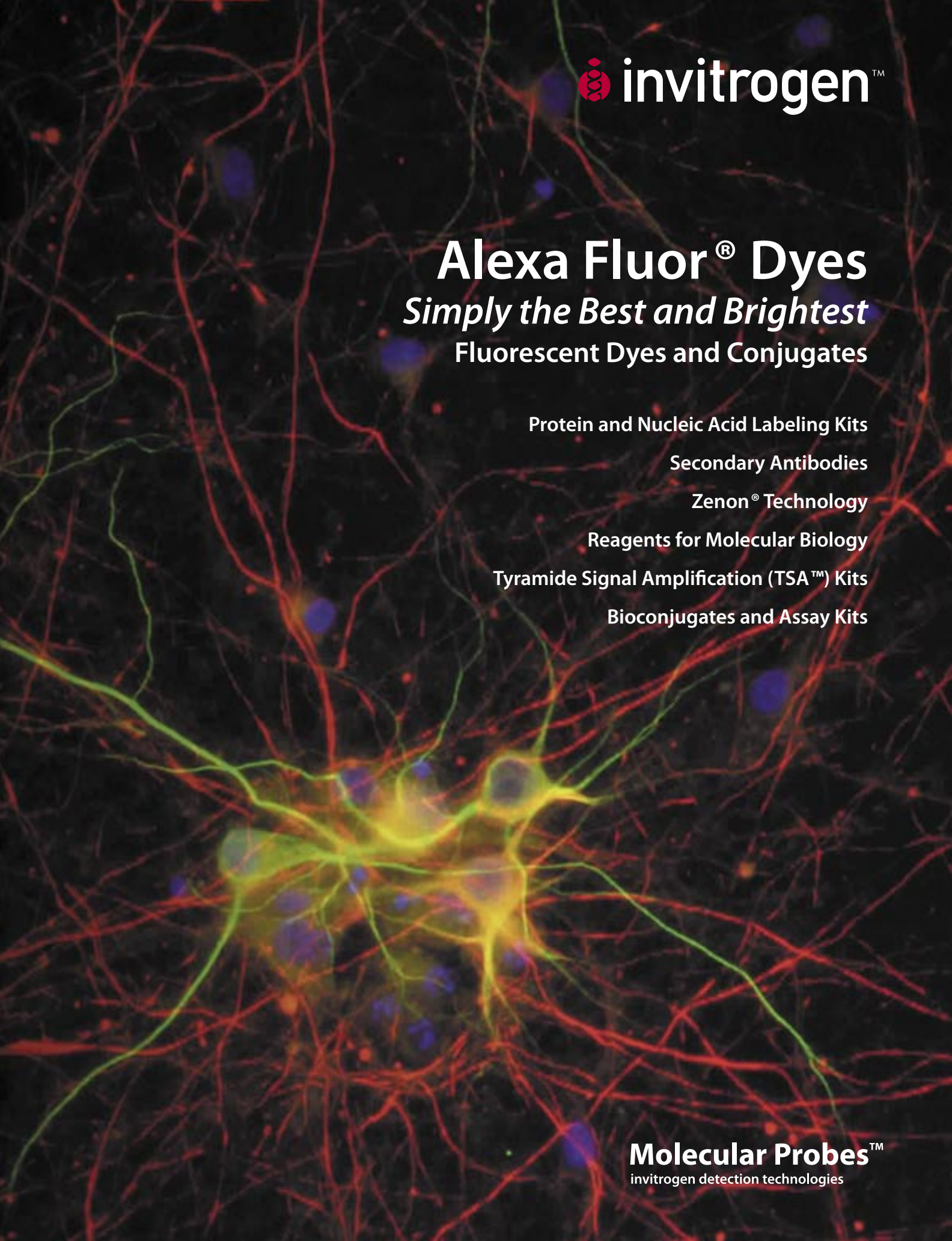


invitrogen™

Alexa Fluor® Dyes

Simply the Best and Brightest

Fluorescent Dyes and Conjugates

Protein and Nucleic Acid Labeling Kits

Secondary Antibodies

Zenon® Technology

Reagents for Molecular Biology

Tyramide Signal Amplification (TSA™) Kits

Bioconjugates and Assay Kits

Molecular Probes™
invitrogen detection technologies

The Alexa Fluor Dye Series

Peak Performance across the Visible Spectrum and Beyond

The Alexa Fluor dyes—a series of superior fluorescent dyes that spans the near-UV, visible, and near-IR spectra (Table 1, Figure 1)—represent a major breakthrough in the development of fluorescent labeling reagents. These dyes, without exception, produce the best and brightest conjugates we have ever tested. Dyes ranging from the blue to the far red provide many options for multicolor detection and fluorescence resonance energy transfer (FRET). Tandem conjugates of the Alexa Fluor dyes with R-phycoerythrin or allophycocyanin further expand the utility of the Alexa Fluor dyes in multicolor applications. Benefits of the Alexa Fluor dyes and their conjugates include the following:

Brightness

Alexa Fluor conjugates exhibit more intense fluorescence than other spectrally similar conjugates.

Photostability

Alexa Fluor conjugates are more photostable than most other fluorescent conjugates, allowing more time for image capture.

Instrument compatibility

Absorption spectra of the Alexa Fluor conjugates are matched to the principal output wavelengths of common excitation sources.

Color selection

Alexa Fluor dye conjugates are available in several distinct fluorescent colors, ranging from blue to far red.

Water solubility

Alexa Fluor reactive dyes have good water solubility, so conjugations can be performed without organic solvents, and the conjugates are relatively resistant to precipitation during storage.

pH insensitivity

Alexa Fluor dyes remain highly fluorescent over a broad pH range.

Table 1. Spectral characteristics of the Alexa Fluor dyes.

Color		Alexa Fluor Dye	Abs*	Em*	Extinction Coefficient†
1		Alexa Fluor 350	346	442	19,000
2		Alexa Fluor 405	401	421	34,000
3		Alexa Fluor 430	433	541	16,000
4		Alexa Fluor 488	495	519	71,000
5		Alexa Fluor 514	517	542	80,000
6		Alexa Fluor 532	532	553	81,000
7		Alexa Fluor 546	556	573	104,000
8		Alexa Fluor 555	555	565	150,000
9		Alexa Fluor 568	578	603	91,300
10		Alexa Fluor 594	590	617	73,000
11		Alexa Fluor 610	612	628	138,000
12		Alexa Fluor 633	632	647‡	100,000
13		Alexa Fluor 647	650	665‡	239,000
14		Alexa Fluor 660	663	690‡	132,000
15		Alexa Fluor 680	679	702‡	184,000
16		Alexa Fluor 700	702	723‡	192,000
17		Alexa Fluor 750	749	775‡	240,000

* Absorbance and fluorescence emission maxima, in nm. † Extinction coefficient at λ_{max} in $cm^{-1}M^{-1}$. ‡ Human vision is insensitive to light beyond ~650 nm, and therefore it is not possible to view the far-red–fluorescent dyes by looking through the eyepiece of a conventional fluorescent microscope. Colors in this table represent the emission colors in the spectra in Figure 1.

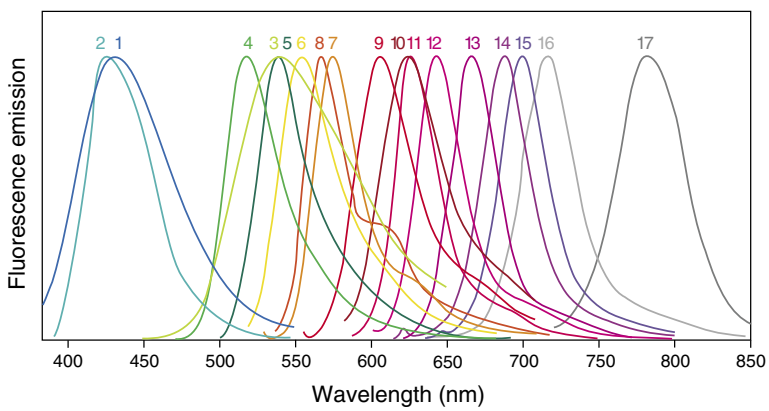
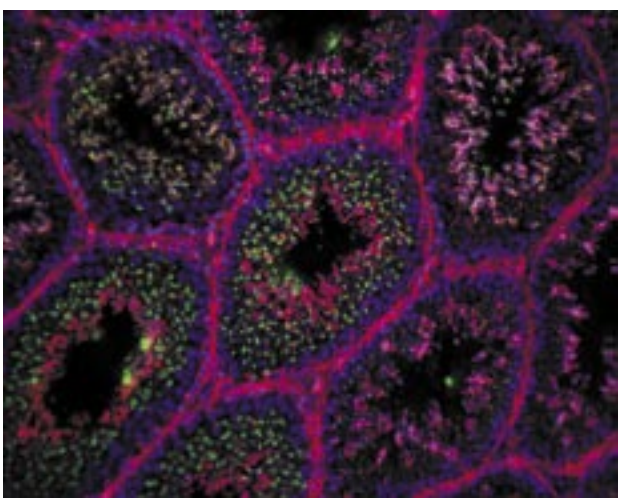
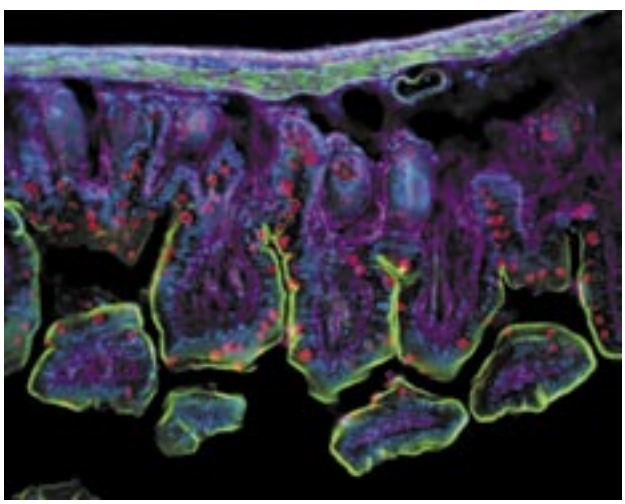


Figure 1. Emission spectra for the Alexa Fluor dye series.

Contents



Mouse intestine section. Actin was labeled with Alexa Fluor 488 phalloidin (green), fibronectin was labeled with an anti-fibronectin antibody and visualized with an Alexa Fluor 647 goat anti–chicken IgG (pseudocolored purple), goblet cells were labeled with an Alexa Fluor 594 conjugate of wheat germ agglutinin (red), and nuclei were labeled with anti–cdc6 peptide antibody and visualized with an Alexa Fluor 405 goat anti–mouse IgG (blue).



Mouse intestine section labeled with four fluorescent dyes. Filamentous actin was labeled with Alexa Fluor 488 phalloidin (green), fibronectin was labeled with anti-fibronectin antibody and visualized using Alexa Fluor 647 goat anti–chicken IgG (pseudocolored purple), goblet cells were labeled with an Alexa Fluor 594 conjugate of wheat germ agglutinin (red), and nuclei were labeled with anti–cdc6 peptide antibody and visualized using Alexa Fluor 405 goat anti–mouse IgG (blue).

The Alexa Fluor Dye Series—Peak Performance across the Visible Spectrum

Alexa Fluor 350 Dye—Bright Blue and UV Light Excitable

With its intense blue fluorescence, Alexa Fluor 350 dye is the perfect choice if you want a UV light excitable label. The Alexa Fluor 350 dye, a sulfonated coumarin derivative, is more water soluble than either AMCA or AMCA-X and yields protein conjugates that are more fluorescent than those prepared from its nonsulfonated analog, AMCA. Furthermore, because Alexa Fluor 350 conjugates have slightly shorter wavelength emission maxima than AMCA or AMCA-X conjugates (442 nm versus 448 nm), the fluorescence of Alexa Fluor 350 conjugates is better separated from that of commonly used green fluorophores.

Alexa Fluor 405 Dye—The Optimal Visible Light–Excitable Blue Fluorophore

Protein conjugates prepared with the Alexa Fluor 405 dye excite at 401 nm and emit at 421 nm, making this an optimal dye for 400–410 nm excitation sources, including the 405 nm spectral line of the krypton laser. With its visible-wavelength excitation, the blue-fluorescent Alexa Fluor 405 dye is potentially brighter than UV light–excitable blue fluorophores, whose signal can be obscured by autofluorescence. Furthermore, the Alexa Fluor 405 dye is an ideal label for multicolor applications due to its minimal spectral overlap with green fluorophores.

Alexa Fluor 430 Dye—Absorption at 430 nm with a High Stokes Shift

Few reactive dyes that absorb between 400 nm and 450 nm have appreciable fluorescence beyond 500 nm in aqueous solution. Our Alexa Fluor 430 dye fills this spectral gap. Excitation near its absorption maximum at ~430 nm is accompanied by strong emission near 540 nm.

Alexa Fluor 488 Dye—The Best Green Fluorophore

Protein conjugates prepared with the Alexa Fluor 488 dye are far superior to conjugates of fluorescein, and are indeed much better than conjugates of any other green fluorophore that we have tested, including those of the Cy2 dye. Not only are Alexa Fluor 488 conjugates significantly brighter than fluorescein conjugates, they are *much* more photostable (Figure 2). Also, fluorescence of the Alexa Fluor 488 fluorophore is independent of pH from 4 to 10 (Figure 3). This pH insensitivity is a major improvement over fluorescein, which emits fluorescence that is significantly affected by pH.

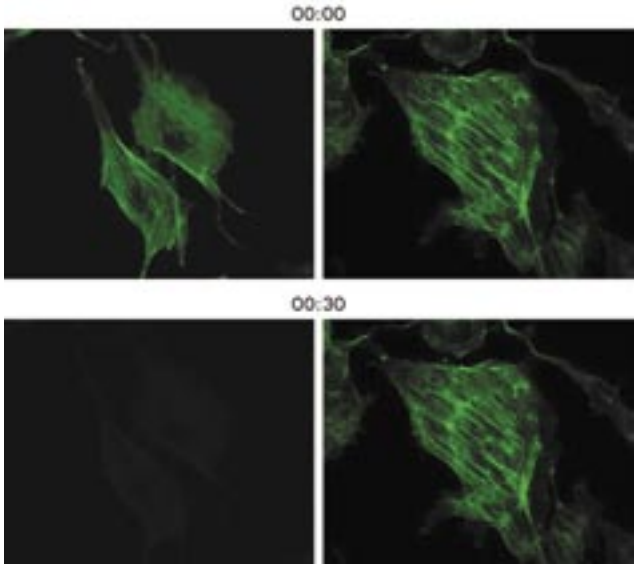


Figure 2. Bovine pulmonary artery endothelial cells were labeled with fluorescein phalloidin (left panels) or Alexa Fluor 488 phalloidin (right panels), which labels filamentous actin, and mounted in PBS. The cells were placed under constant illumination on the microscope, and images were acquired at 1 second intervals for 30 seconds. Under these illumination conditions, fluorescein photobleached to about 20% of its initial value in 30 seconds while the fluorescence of Alexa Fluor 488 phalloidin stayed at essentially the initial value.

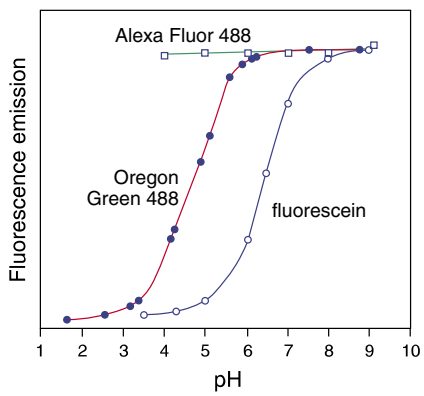


Figure 3. Comparison of pH-dependent fluorescence of the Oregon Green 488 (●), carboxy-fluorescein (○) and Alexa Fluor 488 (□) fluorophores. Fluorescence intensities were measured for equal concentrations of the three dyes using excitation/emission at 490/520 nm.

Alexa Fluor 514 Dye—
A Distinctive Green Fluorophore

The Alexa Fluor 514 dye, with emission profiles visually similar to but spectrally distinct from the Alexa Fluor 488 dye, was developed to increase multiplexing capabilities using instruments such as the Zeiss LSM 510 META and the Leica TCS SP2, which collect spectral distribution information (Figure 4). Like the Alexa Fluor 488 dye, the green-fluorescent Alexa Fluor 514 dye is far superior to fluorescein in both brightness and photostability.

Alexa Fluor 532 Dye—
The Optimal Dye for 532 nm Excitation Sources

With excitation and emission spectra intermediate between those of the green-fluorescent Alexa Fluor 488 dye and orange-fluorescent Alexa Fluor 546 dye, the Alexa Fluor 532 dye and its conjugates are ideal for use with 532 nm excitation sources, including the frequency-doubled Nd:YAG laser.

Alexa Fluor 546 Dye—A More Fluorescent
Alternative to Cy3 Dye and Tetramethylrhodamine

Conjugates prepared with the Alexa Fluor 546 dye are perfect for applications that require fluorescent probes that emit in the orange region of the spectrum. These intensely fluorescent conjugates outperform conjugates of tetramethylrhodamine (TRITC and TAMRA) and Cy3 dye and are readily excited by the strong 546 nm emission of mercury-arc lamps and the 543 nm spectral line of the He–Ne laser.

Alexa Fluor 555 Dye—
A Superior Alternative to the Cy3 Dye

Spectra of the Alexa Fluor 555 conjugates virtually match those of the Cy3 dye, resulting in an optimal match to filters designed for that dye. However, total fluorescence of Alexa Fluor 555 conjugates is higher (Figure 5). The Alexa Fluor 555 dye is also more photostable, providing researchers with additional time for image capture.

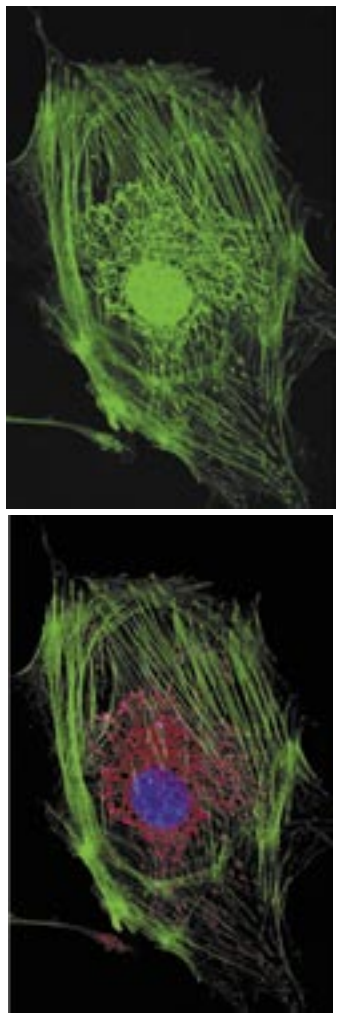


Figure 4. Muntjac fibroblast labeled with three green fluorophores, as seen through a standard microscope filter (top) and after processing with imaging software capable of spectrally unmixing the signals (bottom). The nucleus was labeled with SYTOX Green nucleic acid stain (pseudocolored blue); F-actin was labeled with BODIPY FL phallacidin (green); and mitochondria were labeled with a mouse anti–OxPhos Complex V inhibitor protein monoclonal IgG_1 antibody and visualized with Alexa Fluor 514 goat anti–mouse IgG antibody (pseudocolored red).

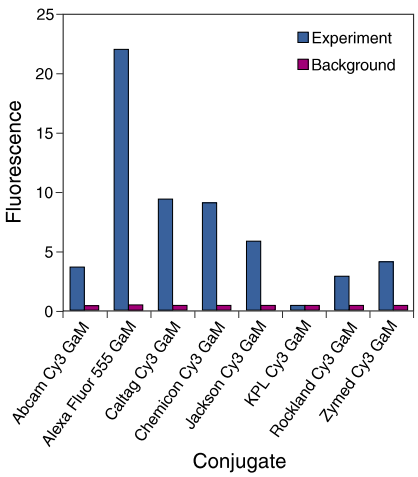


Figure 5. Brightness comparison of Molecular Probes Alexa Fluor 555 goat anti–mouse IgG antibody with Cy3 goat anti–mouse IgG antibody conjugates commercially available from several other companies. Human blood was blocked with normal goat serum and incubated with an anti-CD3 mouse monoclonal antibody; cells were washed, resuspended and incubated with either Alexa Fluor 555 or Cy3 goat anti–mouse IgG antibody at equal concentrations. Red blood cells were lysed, and the samples were analyzed by flow cytometry.

Alexa Fluor 568 Dye—Perfect for 568 nm Excitation Sources

The red-orange–fluorescent Alexa Fluor 568 dye is optimally excited by the 568 nm spectral line of the Ar–Kr mixed-gas laser used in many confocal laser-scanning microscopes. Alexa Fluor 568 conjugates are considerably brighter than Lissamine Rhodamine B conjugates or even Rhodamine Red-X conjugates, which have similar excitation and emission maxima.

Alexa Fluor 594 Dye—A Superior Alternative to the Texas Red Dye

Conjugates prepared with the Alexa Fluor 594 dye emit in the red region of the spectrum, making them particularly useful for multilabeling experiments in combination with green-fluorescent probes. Alexa Fluor 594 conjugates are efficiently excited by the 594 nm line of the orange He–Ne laser. They also are much more fluorescent than Texas Red conjugates and exhibit high photostability (Figure 6).

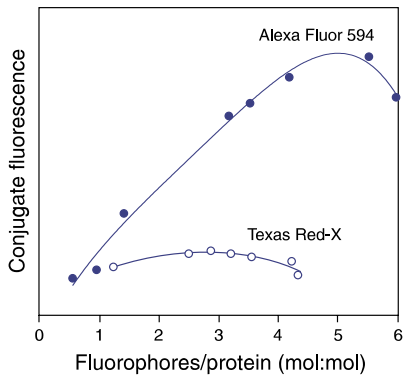


Figure 6. Comparison of the relative fluorescence of Alexa Fluor 594 and Texas Red-X goat anti–mouse IgG antibody $F(ab')_2$ fragment conjugates at various dye/protein ratios.

Alexa Fluor 610 Dye—The Red Jewel of the Alexa Fluor Dye Series

Our bright and photostable Alexa Fluor 610 dye emits an intense red fluorescence that can be visualized with the same optics used for the Texas Red and Alexa Fluor 594 dyes. Unlike the Alexa Fluor 633 dye and longer-wavelength fluorophores, the Alexa Fluor 610 dye can also be seen with the human eye. Previously, this red fluorophore was only available as the acceptor dye in our FRET-based Alexa Fluor dye–phycobiliprotein tandem conjugates. With excitation/emission maxima of ~612/628 nm, the Alexa Fluor 610 dye can be easily differentiated from green fluorophores.

Alexa Fluor 633 Dye—A Preferred Dye for the 633 nm He–Ne Laser Line

Far-red–fluorescent dyes are among the most sought-after labels for fluorescence imaging because their spectra are well beyond the range of most sample autofluorescence. The growing popularity of the 633 nm spectral line of the He–Ne laser and the 635 nm spectral line of red diode lasers prompted us to create compatible dyes. Alexa Fluor 633 conjugates are bright and photostable, with peak absorption centered at 632 nm and peak emission centered at 650 nm.

Alexa Fluor 647 Dye—A Superior Alternative to the Cy5 Dye

Spectra of the Alexa Fluor 647 conjugates virtually match those of the Cy5 dye, resulting in an optimal match to optical filters designed for that dye. However, total fluorescence of the secondary antibody conjugates of the Alexa Fluor 647 dye is significantly higher than that of Cy5 conjugates supplied by other companies. Also, unlike the Cy5 dye, the Alexa Fluor 647 dye has very little change in absorption or fluorescence spectra when conjugated to most proteins, oligonucleotides, or nucleic acids, thus yielding greater total fluorescence at the same degree of substitution (Figure 7).

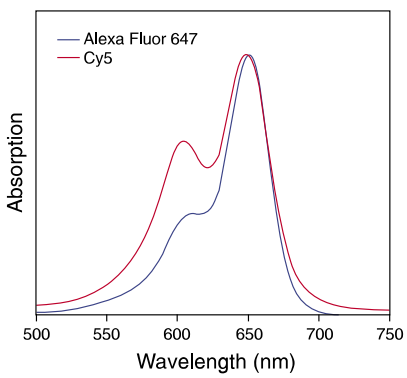


Figure 7. The absorption spectra of the Cy5 dye conjugates of both proteins and nucleic acids show an additional peak at about 600 nm when compared with the spectrum of the free dye. However, light absorbed by the Cy5 dye conjugates at this wavelength does not result in fluorescence. Alexa Fluor 647 dye conjugates of proteins do not exhibit this spectral anomaly. Spectra were normalized to the same peak intensity for comparison purposes.

Alexa Fluor 660 Dye—
An Optimal Dye for the 647 nm Krypton-Ion Laser Line

The Alexa Fluor 660 dye is optimally excited with the 647 nm spectral line of the krypton-ion laser and well excited by the 633 nm spectra line of the He–Ne laser. Protein conjugates of the Alexa Fluor 660 dye produce bright far-red–fluorescence emission, with a peak at 690 nm. The wide separation of its emission from that of other fluorophores allows use of the Alexa Fluor 660 dye with other fluorescent labels, including the Alexa Fluor 545 and Cy3 dyes and phycoerythrin conjugates. The Alexa Fluor 660 dye is the dye of choice as a "second label" with allophycocyanin (APC) conjugates in flow cytometry applications.

Alexa Fluor 680 Dye—
An Alternative to the Cy5.5 Dye

With a peak excitation at 679 nm and maximum emission at 702 nm, the Alexa Fluor 680 dye is spectrally similar to the Cy5.5 dye. Fluorescence emission of the Alexa Fluor 680 dye is well separated from that of other commonly used red fluorophores, such as the tetramethylrhodamine, Texas Red, R-phycoerythrin, Alexa Fluor 594, and Alexa Fluor 647 dyes.

Alexa Fluor 700 Dye—
The Optimal Dye for Far-Red Diode Lasers

With an absorption maximum at 696 nm, the Alexa Fluor 700 dye can be excited with a xenon-arc lamp, far-red diode laser, or dye-pumped laser operating in the 675–700 nm range. The Alexa Fluor 700 dye provides infrared fluorescence emission, with a peak at 719 nm.

Alexa Fluor 750 Dye—
Our Longest-Wavelength Alexa Fluor Dye

Spectrally similar to the Cy7 dye, the Alexa Fluor 750 dye is the longest wavelength Alexa Fluor dye currently available (Figure 8). Its fluorescence emission maximum at 779 nm is well separated from commonly used far-red fluorophores such as Alexa Fluor 647, Alexa Fluor 660, or allophycocyanin (APC), facilitating multicolor analysis. With a peak excitation at ~752 nm, conjugates of the Alexa Fluor 700 dye are well excited by a xenon-arc lamp or dye-pumped lasers operating in the 720–750 nm range.

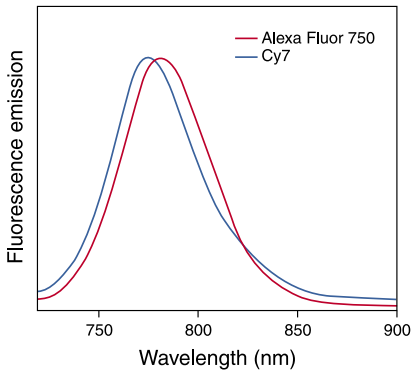


Figure 8. Comparison of the fluorescence emission spectra of the Alexa Fluor 750 and Cy7 dyes. Spectra have been normalized to the same intensity for comparison purposes.

Alexa Fluor Conjugates for Secondary Detection

Bright, Photostable Anti-IgG and Anti-IgM Antibody Conjugates

Molecular Probes provides scientists with an extensive and growing selection of secondary immunoreagents for use in fluorescence microscopy, flow cytometry, microplate-based assays, nucleic acid hybridization assays, and several other techniques. Our **species-specific anti-IgG antibodies** (Tables 2 and 3), which are raised against IgG heavy and light chains, are affinity purified and adsorbed against the sera of a number of species to minimize crossreactivity. The anti-IgM conjugates (Table 4) are prepared from well-characterized antibodies that have been purified by IgM affinity chromatography and react specifically with IgM heavy chains (μ chains).

Table 3. Isotype-specific antibodies.

Alexa Fluor Dye	IgG_1 (γ_1)	IgG_{2a} (γ_{2a})	IgG_{2b} (γ_{2b})	IgG_3 (γ_3)
Alexa Fluor 350	A21120	A21130	A21140	
Alexa Fluor 488	A21121	A21131	A21141	A21151
Alexa Fluor 546	A21123	A21133	A21143	
Alexa Fluor 555	A21127	A21137	A21147	
Alexa Fluor 568	A21124	A21134	A21144	
Alexa Fluor 594	A21125	A21135	A21145	A21155
Alexa Fluor 633	A21126	A21136	A21146	
Alexa Fluor 647	A21240	A21241	A21242	

Table 2. Alexa Fluor secondary antibody conjugates for mouse, rabbit, chicken, guinea pig, hamster, human, rat, sheep, and goat IgG antibodies.

Antibody	Host	Alexa Fluor 350	Alexa Fluor 405	Alexa Fluor 430	Alexa Fluor 488	Alexa Fluor 514	Alexa Fluor 532	Alexa Fluor 546	Alexa Fluor 555	Alexa Fluor 568	Alexa Fluor 594
Anti–mouse IgG	Goat	A11045 A11068 *	A31553	A11063	A11001 A11017 *		A11002	A11003 A11018 *	A21422 A21425 *	A11004 A11019 *	A11005 A11020 *
Anti–mouse IgG (highly cross-adsorbed)	Goat	A21049			A11029			A11030	A21424	A11031	A11032
Anti–mouse IgG	Rabbit	A21062			A11059 A21204 *			A11060	A21427	A11061	A11062 A21205 *
Anti–mouse IgG	Chicken				A21200						A21201
Anti–mouse IgG	Donkey				A21202				A31570		A21203
Anti–rabbit IgG	Goat	A11046 A11069 *†	A31556	A11064	A11008 A11070 *†	A31558	A11009	A11010 A11071 *†	A21428 A21430 *†	A11011 A21069 *†	A11012 A11072 *†
Anti–rabbit IgG (highly cross-adsorbed)	Goat	A21068			A11034			A11035	A21429	A11036	A11037
Anti–rabbit IgG	Chicken				A21441						A21442
Anti–rabbit IgG	Donkey				A21206				A31572		A21207
Anti–chicken IgG	Goat				A11039			A11040	A21437	A11041	A11042
Anti–guinea pig IgG (highly cross-adsorbed)	Goat				A11073			A11074	A21435	A11075	A11076
Anti–hamster IgG	Goat				A21110			A21111		A21112	A21113
Anti–human IgG	Goat				A11013			A21089	A21433	A21090	A11014
Anti–rat IgG	Goat	A21093			A11006			A11081	A21434	A11077	A11007
Anti–rat IgG	Chicken				A21470						A21471
Anti–rat IgG	Donkey				A21208						A21209
Anti–rat IgG	Rabbit				A21210						A21211
Anti–sheep IgG	Donkey	A21097			A11015			A21098	A21436	A21099	A11016
Anti–goat IgG	Rabbit				A11078 A21222 *‡			A21085	A21431	A11079	A11080 A21223 *‡
Anti–goat IgG	Chicken				A21467						A21468
Anti–goat IgG	Donkey	A21081			A11055			A11056	A21432	A11057	A11058

* $F(ab')_2$ fragments. † Human and mouse serum, mouse plasmacytoma/hybridoma proteins, and purified human paraproteins. ‡ Human and mouse serum proteins.

Isotype-Specific Antibodies

Molecular Probes offers **isotype-specific antibodies** to aid in multilabeling experiments (Table 4). The Alexa Fluor goat anti–mouse IgG isotype-specific antibodies have been cross-adsorbed against mouse IgM, IgA, pooled human sera, purified human paraproteins, and other isotypes to minimize crossreactivity.

Alexa Fluor Colloidal Gold and FluoroNanogold Conjugates

The colloidal gold and FluoroNanogold conjugates (Tables 5 and 6, next page) may be used as probes in immunoblotting, light microscopy, fluorescence microscopy, or electron microscopy. Because they include both a fluorescent dye and a gold particle, they can be used for correlated immunofluorescence and electron microscopy in a two-step labeling procedure, instead of the three-step indirect labeling required with conventional nonfluorescent anti-IgG– or streptavidin–colloidal gold complexes. The FluoroNanogold particle is smaller than the colloidal gold particle and thus better for fine electron microscopy imaging. The colloidal gold conjugates are also available as 5 nm and 10 nm streptavidin conjugates.

Table 4. Anti-IgM conjugates.

Dye	Anti-Mouse	Anti-Rat	Anti-Human
Alexa Fluor 350	A31552		
Alexa Fluor 488	A21042	A21212	A21215
Alexa Fluor 546	A21045		
Alexa Fluor 555	A21426		
Alexa Fluor 568	A21043		
Alexa Fluor 594	A21044	A21213	A21216
Alexa Fluor 633	A21046		
Alexa Fluor 647	A21238	A21248	A21249
Alexa Fluor 680	A21048		

Alexa Fluor 610	Alexa Fluor 633	Alexa Fluor 635	Alexa Fluor 647	Alexa Fluor 660	Alexa Fluor 680	Alexa Fluor 700	Alexa Fluor 750	Adsorbed against
A31550	A21050 A21053 *	A31574	A21235 A21237 *	A21054	A21057 A21059 *	A21036	A21037	Human IgG and serum
	A21052	A31575	A21236	A21055	A21058			Bovine, goat, rabbit, rat, and human IgG and human serum
	A21063		A21239		A21065			Human serum
			A21463					Human and rabbit IgG
			A31571					Bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat, and sheep serum proteins
A31551	A21070 A21072 *†	A31576	A21244 A21246 *†	A21073	A21076 A21077 *†	A21038	A21039	Human and mouse IgG, human and bovine sera
	A21071	A31577	A21245	A21074	A21109			Bovine, goat, mouse, rat, and human IgG
			A21443					Human and mouse IgG
			A31573					Bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat, and sheep serum proteins
	A21103		A21449					
	A21105		A21450					Bovine, chicken, goat, hamster, human, mouse, rabbit, rat, and sheep sera
			A21451					Mouse and rat IgG
	A21091		A21445					Mouse, rabbit, and bovine sera
	A21094		A21247		A21096			Mouse IgG and mouse and human sera
			A21472					Human and rabbit IgG
								Bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rabbit, and sheep serum proteins
								Human IgG
	A21100		A21448		A21102			Human IgG and mouse, rabbit, bovine, and human sera
	A21086		A21446		A21088			Human and rat serum proteins
			A21469					Human, mouse, and rabbit IgG
	A21082		A21447	A21083	A21084			Rabbit, mouse, rat, and human IgG

Table 5. Alexa Fluor 488 colloidal gold antibody conjugates.

Diameter	Goat Anti–Mouse IgG	Goat Anti–Rabbit IgG	Streptavidin
5 nm	A31560	A31565	A32360
10 nm	A31561	A31566	A32361

Table 6. Alexa Fluor FluoroNanogold conjugates.

Dye	Fab′ Fragment of Goat Anti–Mouse IgG	Fab′ Fragment of Goat Anti–Rabbit IgG	Fab′ Fragment of Rabbit Anti–Goat IgG
Alexa Fluor 488	A24920	A24922	A24924
Alexa Fluor 594	A24921	A24923	A24925

Perform Three-, Four-, or Five-Color Analyses by Flow Cytometry with a Single Excitation—Alexa Fluor Tandem Conjugates of Phycobiliproteins

With efficient excitation at 488 nm and emission at 578 nm, the phycobiliprotein R-phycoerythrin (R-PE) is often used in combination with green-fluorescent detection reagents like fluorescein (FITC), Oregon Green 488, or Alexa Fluor 488 dye to detect two different signals using simultaneous excitation with the 488 nm spectral line. By conjugating R-PE to longer wavelength light–emitting fluorescence acceptors, an energy transfer cascade is established wherein excitation of the R-PE produces fluorescence of the acceptor dye by the process of fluorescence resonance energy transfer (FRET). This process can be quite efficient, resulting in almost total transfer of energy from the phycobiliprotein to the acceptor dye of these "tandem conjugates."

We have conjugated R-PE with four of our Alexa Fluor dyes—the Alexa Fluor 610, Alexa Fluor 647, Alexa Fluor 680, and Alexa Fluor 750 dyes—then attached these fluorescent proteins to antibodies, streptavidin, or our Zenon labeling reagents to yield tandem

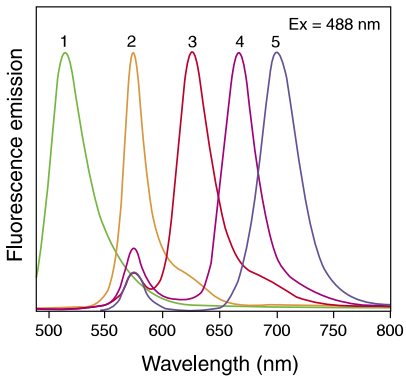


Figure 9. Normalized fluorescence emission spectra of 1) Alexa Fluor 488 goat anti–mouse IgG antibody, 2) R-phycoerythrin goat anti–mouse IgG antibody, 3) Alexa Fluor 610–R-phycoerythrin goat anti–mouse IgG antibody, 4) Alexa Fluor 647–R-phycoerythrin goat anti–mouse IgG antibody, and 5) Alexa Fluor 680–R-phycoerythrin goat anti–mouse IgG antibody. The tandem conjugates permit simultaneous multicolor labeling and detection of up to five targets with excitation by a single excitation source—the 488 nm spectral line of the argon-ion laser.

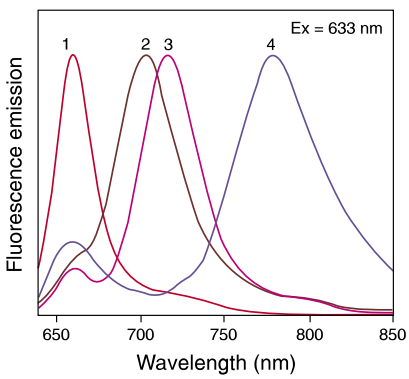


Figure 11. Normalized fluorescence emission spectra of 1) allophycocyanin, crosslinked, goat anti–mouse IgG antibody, 2) Alexa Fluor 680–allophycocyanin goat anti–mouse IgG antibody, 3) Alexa Fluor 700–allophycocyanin goat anti–mouse IgG antibody, and 4) Alexa Fluor 750–allophycocyanin goat anti–mouse IgG antibody. The tandem conjugates permit simultaneous multicolor labeling and detection of up to three targets with excitation by a single excitation source—the 633 nm spectral line of the He–Ne laser.

Table 7. Tandem conjugates of R-phycoerythrin (R-PE).

Acceptor Dye	Ex/Em*	Tandem Conjugate			
		Anti–Mouse IgG†	Anti–Rabbit IgG†	Streptavidin	Zenon Alexa Fluor Mouse IgG_1 Labeling Kit‡
Alexa Fluor 610	496, 565/627	A20980	A20981	S20982	Z25020
Alexa Fluor 647	496, 565/667	A20990	A20991	S20992	Z25021
Alexa Fluor 680	496, 565/702	A20983	A20984	S20985	Z25022
Alexa Fluor 750	496, 565/791			S32363	

* Fluorescence excitation and emission maxima, in nm. † Host = goat. ‡ See page 12 for more information on our Zenon antibody labeling technology.

Table 8. Tandem conjugates of allophycocyanin (APC).

Acceptor Dye	Ex/Em*	Tandem Conjugate			
		Anti–Mouse IgG†	Anti–Rabbit IgG†	Streptavidin	Zenon Alexa Fluor Mouse IgG_1 Labeling Kit‡
Alexa Fluor 680	650/702	A21000	A21001MP	S21002	
Alexa Fluor 700	650/719			S21005	Z25030
Alexa Fluor 750	650/779	A21006		S21008	Z25031

* Fluorescence excitation and emission maxima, in nm. † Host = goat. ‡ See page 12 for more information on our Zenon antibody labeling technology.

constructs that can be excited with the 488 nm spectral line of the argon-ion laser (Table 7, Figure 9). These tandem constructs can potentially be used for simultaneous three-, four-, or five-color labeling with a single excitation (Figure 10).

We have also conjugated APC to our Alexa Fluor 680, Alexa Fluor 700, and Alexa Fluor 750 dyes to provide tandem conjugates that can be excited by the He–Ne laser at 633 nm or by the krypton-ion laser at 647 nm (Table 8, Figure 11). These Alexa Fluor dye–APC tandem conjugates can potentially be combined with direct APC conjugates for simultaneous three- or four-color applications.

Alexa Fluor Conjugates of Protein A and Protein G for Immunodetection

Protein A and protein G are bacterial proteins that bind with high affinity to the Fc portion of various classes and subclasses of immunoglobulins from a variety of species and can be used as alternatives to species-specific anti-IgG antibodies. We offer **Alexa Fluor conjugates of protein A** in four colors (Table 9) as well as an **Alexa Fluor 488 protein G conjugate** (P11065).

Highly Sensitive Detection of Biotinylated Targets

Avidin, streptavidin, and NeutrAvidin biotin-binding protein each bind four biotins per protein molecule with high affinity and selectivity. Fluorescent streptavidin is extensively used in DNA hybridization techniques, immunohistochemistry, and multicolor flow cytometry. Our **Alexa Fluor streptavidin conjugates** are available in 18 different colors (Table 10, next page, Figures 12 and 13) as well as tandem conjugates with R-PE and APC (Tables 7 and 8). We also offer an **Alexa Fluor 488 avidin conjugate** (A21370) and an **Alexa Fluor 350 conjugate of NeutrAvidin biotin-binding protein** (A11236). The enhanced brightness and photostability

Table 9. Alexa Fluor conjugates of protein A.

Dye	Catalog Number
Alexa Fluor 488	P11047
Alexa Fluor 546	P11049
Alexa Fluor 594	P11051
Alexa Fluor 647	P21462

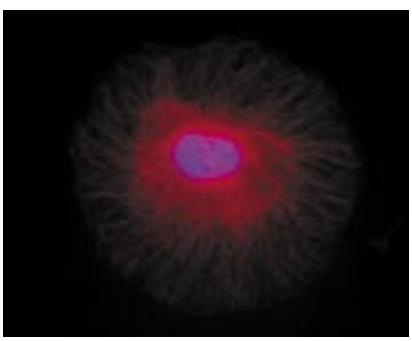


Figure 12. Formaldehyde-fixed and permeabilized bovine pulmonary artery endothelial cell labeled with the biotin-XX conjugate of anti–α-tubulin and Alexa Fluor 568 streptavidin. The nucleus was stained with DAPI and the sample was then mounted using the reagents in the ProLong Antifade Kit.

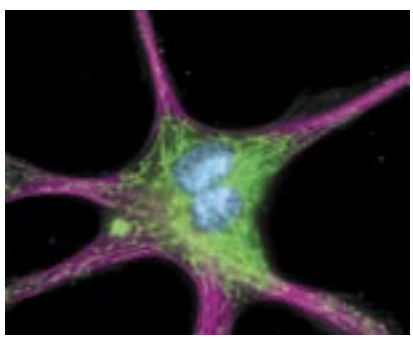


Figure 13. The cytoskeleton of a fixed and permeabilized bovine pulmonary artery endothelial cell detected using mouse monoclonal anti–α-tubulin antibody, visualized with Alexa Fluor 647 goat anti–mouse IgG antibody, and pseudocolored magenta. Endogenous biotin in the mitochondria was labeled with green-fluorescent Alexa Fluor 488 streptavidin, and DNA was stained with blue-fluorescent DAPI.

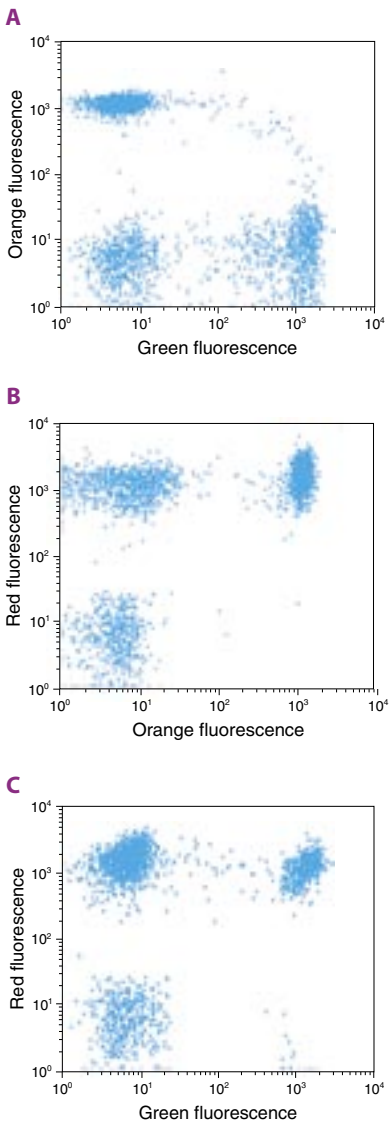


Figure 10. Simultaneous detection of three cell surface markers using an Alexa Fluor 610–R-phycoerythrin tandem conjugate, Alexa Fluor 488 dye, and R-phycoerythrin labels. Lymphocytes from ammonium chloride RBC–lysed whole blood were labeled with a biotinylated mouse anti–human CD3 monoclonal antibody, with Alexa Fluor 610–R-phycoerythrin tandem dye–labeled streptavidin, and with directly conjugated primary antibodies against the CD8 and CD4 markers. Labeling was analyzed by flow cytometry. The bivariate scatter plots show the expected mutually exclusive populations of CD4 and CD8 positive cells (panel A), together with co-positive CD3/CD4 (panel B) and CD3/CD8 (panel C) populations.

of these Alexa Fluor conjugates make them ideal for even the most demanding imaging applications. Or, if you prefer to use an anti-biotin antibody to detect your biotinylated targets, we also offer an **anti-biotin antibody** (mouse monoclonal 2F5) conjugated to **Alexa Fluor 488** or **Alexa Fluor 594 dye** (Table 11).

Anti-Dye and Anti-Hapten Antibodies

In addition to being useful for direct optical detection, some fluorogenic and chromogenic dyes make excellent haptens that can be recognized by secondary detection reagents in applications such as *in situ* hybridization, enzyme-linked immunosorbent assay (ELISA) techniques, and detection of labeled targets on blots. They can also be used to amplify the fluorescent signal (see Alexa Fluor Signal-Amplification Kits, page 15) or change the fluorescence to a different color (Figure 14). Molecular Probes offers several **anti-dye and anti-hapten antibodies** (Table 11).

Create Bright and Photostable GFP Signals

Expression of the intrinsically fluorescent green-fluorescent protein (GFP) from the jellyfish *Aequorea victoria* has become a popular method for following gene expression and protein localization. Researchers can now combine the brightness and photostability of an Alexa Fluor dye with this enabling technology by using one of our direct conjugates of a rabbit antibody that is raised against GFP purified directly from *A. victoria*. Bright and photostable green-fluorescent images are easily achieved with our **Alexa Fluor 488 conjugate of anti-GFP**. The **Alexa Fluor 555, Alexa Fluor 594,** and **Alexa Fluor 647 conjugates of anti-GFP** (Table 12 and Figure 15) will convert the green fluorescence to orange, red, and far red, respectively, allowing an additional green fluorophore to be used in the application.

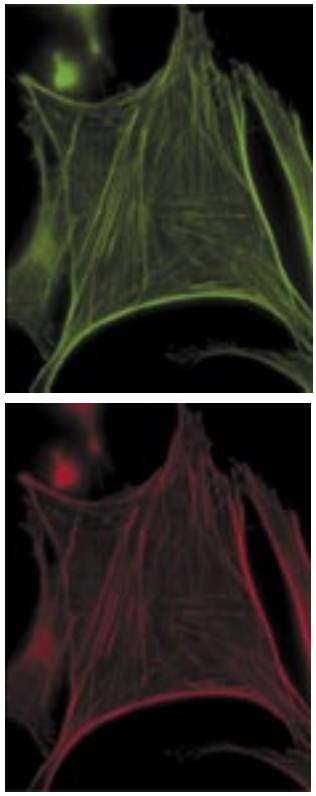


Figure 14. Fixed and permeabilized bovine pulmonary artery endothelial cells were labeled with the filamentous actin (F-actin) stain fluorescein phalloidin (top). An Alexa Fluor 594 anti–fluorescein/Oregon Green rabbit IgG antibody converted the green fluorescence to red (bottom).

Table 10. Alexa Fluor streptavidin conjugates.

Dye	Catalog Number
Alexa Fluor 350	S11249
Alexa Fluor 405	S32351
Alexa Fluor 430	S11237
Alexa Fluor 488	S11223, S32354 *
Alexa Fluor 514	S32353
Alexa Fluor 532	S11224
Alexa Fluor 546	S11225
Alexa Fluor 555	S21381, S32355 *
Alexa Fluor 568	S11226
Alexa Fluor 594	S11227, S32356 *
Alexa Fluor 610	S32359
Alexa Fluor 633	S21375
Alexa Fluor 635	S32364
Alexa Fluor 647	S21374, S32357 *
Alexa Fluor 660	S21377
Alexa Fluor 680	S21378, S32358 *
Alexa Fluor 700	S21383
Alexa Fluor 750	S21384

* Supplied as 0.5 ml of a 2 mg/ml solution. All other conjugates supplied as 1 mg units.

Table 11. Anti-dye and anti-hapten antibodies.

Target	Antibody	Dye	Catalog Number
Fluorescein/Oregon Green	Rabbit IgG	Alexa Fluor 488	A11090
Fluorescein/Oregon Green	Goat IgG	Alexa Fluor 488	A11096
Fluorescein/Oregon Green	Rabbit IgG	Alexa Fluor 594	A11091
Alexa Fluor 488	Rabbit IgG	NA	A11094
DNP-KLH *	Rabbit IgG	Alexa Fluor 488	A11097
Biotin	Mouse IgG	Alexa Fluor 488	A31801
Biotin	Mouse IgG	Alex Fluor 594	A31800

* Our anti-DNP antibody is prepared against DNP–keyhole limpet hemocyanin (DNP-KLH); thus, the antibody and its conjugates do not crossreact with BSA, a common blocking reagent in hybridization applications. NA = Not applicable.

Table 12. Alexa Fluor dye–labeled rabbit anti-GFP conjugates.

Dye	Catalog Number
Alexa Fluor 488	A21311
Alexa Fluor 555	A31851
Alexa Fluor 594	A21312
Alexa Fluor 647	A31852

Figure 15. NIH 3T3 cells were transiently transfected with a green-fluorescent protein (GFP) expression vector, then plated and allowed to attach and proliferate. The cells were fixed and labeled with our Alexa Fluor 594 conjugate of the anti-GFP antibody. About 10% of the cells were expressing GFP and show dual labeling of both GFP (green fluorescence) and the anti-GFP antibody (red fluorescence). In this overlay of fluorescence and differential interference contrast (DIC) micrographs, the GFP-transfected cells exhibit green and red signals that overlap to yield yellow, and DAPI stains the nuclei with a light-blue fluorescence. In the cells that are not transfected, the DAPI-stained nuclei exhibit a bright blue fluorescence.

Table 13. Image-iT FX Kits.

Dye	Goat Anti–Mouse IgG	Goat Anti–Rabbit IgG	Streptavidin
Alexa Fluor 350	I37150 (Kit #1)	I37155 (Kit #6)	I37160 (Kit #11)
Alexa Fluor 488	I37151 (Kit #2)	I37156 (Kit #7)	I37161 (Kit #12)
Alexa Fluor 555	I37152 (Kit #3)	I37157 (Kit #8)	I37162 (Kit #13)
Alexa Fluor 594	I37153 (Kit #4)	I37158 (Kit #9)	I37163 (Kit #14)
Alexa Fluor 647	I37154 (Kit #5)	I37159 (Kit #10)	I37164 (Kit #15)

Image-iT FX Kits for Optimal Fixed-Cell Imaging

The Image-iT FX Kits (Table 13) make high-quality, information-packed images easy to obtain. These new kits provide the best secondary detection reagents and tools for optimal fluorescence signals:

- Alexa Fluor conjugates for superior photostability and brightness
- ProLong Gold antifade reagent for reduced photobleaching
- Image-iT FX signal enhancer for improved signal-to-noise ratio

ProLong Gold Reagent—Ready-to-Use Antifade Reagent

ProLong Gold antifade reagent (P36930) is an improved version of the very effective ProLong antifade reagent. ProLong Gold reagent is premixed and ready to use—just add a drop to your preparation and mount. ProLong Gold reagent outperforms most other commercially available antifade reagents, significantly reducing fluorophore photobleaching while causing little or no quenching of the fluorescence signal (Figure 16). This reagent offers excellent compatibility with a multitude of dyes and dye complexes, making it an especially valuable tool for multicolor applications. ProLong Gold reagent cures within 24 hours, and the sample can be saved for months after mounting.

Image-iT FX Signal Enhancer—Eliminate Background Staining

Image-iT FX signal enhancer (I36933) dramatically improves the signal-to-noise ratio of immunolabeled cells and tissues, allowing you to clearly visualize targets that would normally be indistinguishable due to background fluorescence. Background staining typically seen with fluorescent dyes is largely eliminated when Image-iT FX signal enhancer is applied to fixed and permeabilized cells before staining (Figure 17).

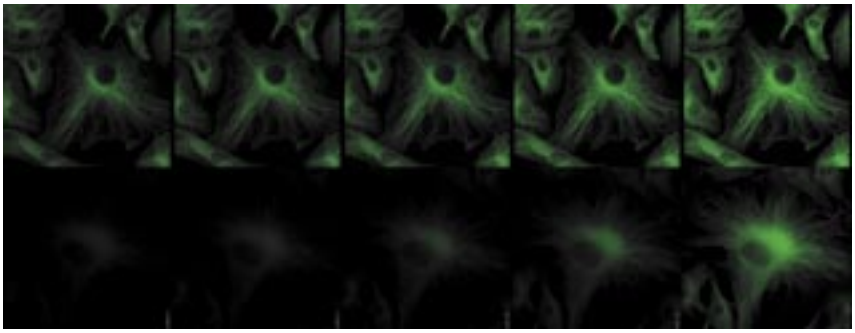


Figure 16. A 20 second time series showing enhanced resistance to photobleaching afforded by ProLong Gold antifade reagent. Fixed bovine pulmonary artery endothelial cells were labeled with anti–α-tubulin and visualized with fluorescein goat anti–mouse IgG antibody. The samples were mounted in ProLong Gold antifade reagent (top) or PBS (bottom). Images were acquired at 5 second intervals using a 40×/1.3 NA oil immersion objective with continuous illumination from a standard 100 watt Hg-arc lamp.

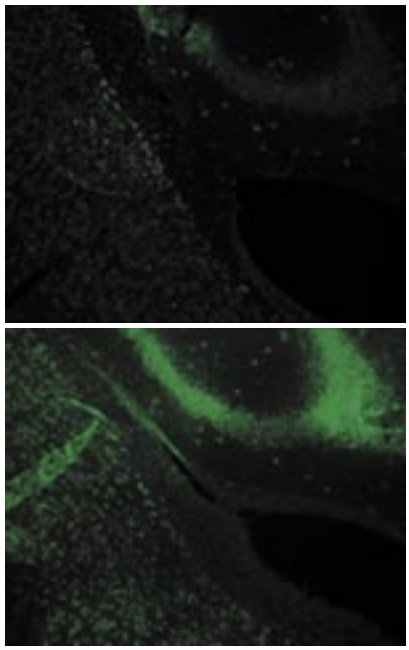


Figure 17. Reduced background staining afforded by Image-iT FX signal enhancer. Mouse brain cryosections were permeabilized and antigen retrieval was carried out. The sections were then treated for 30 minutes with Image-iT FX signal enhancer (top) or left untreated (bottom). Sections were labeled with the neural cell body selective antibody anti–Hu C/D and visualized using TSA Kit #2 with the HRP conjugate of goat anti–mouse IgG and Alexa Fluor 488 tyramide. Sections were mounted using the reagents in the ProLong Antifade Kit.

A New Approach to Immunolabeling—Zenon Antibody Labeling Technology

Our patent-pending Zenon labeling technology provides a versatile and easy-to-use method for labeling mouse and rabbit antibodies, even with very small (submicrogram) amounts of starting material (Figure 18). The Zenon antibody labeling technology forms a labeling complex using a fluorophore-labeled Fab fragment that is selective for the Fc portion of a primary antibody. Simple mixing of the labeled Fab fragment with an intact primary antibody rapidly and quantitatively forms the labeling complex. This labeling complex is then used for staining in the same manner as a covalently labeled primary antibody. Advantages of the Zenon technology include:

Speed—The entire labeling procedure takes only 10 minutes.

Efficiency—Nearly 100% of the antibody is labeled.

Economy—Submicrogram amounts of a primary antibody can be efficiently labeled.

Simplicity—No pre- or postlabeling purification of the antibody is required.

Flexibility—Multiple primary antibodies of the same isotype can easily be used in a single experiment (Figure 19).

Compatibility—Labeled antibody complexes can be used in flow cytometry, imaging, and high-throughput screening—anywhere a labeled primary antibody is suitable.

The fluorescence intensity and utility of Zenon labeling complexes of primary antibodies are similar to the properties of directly conjugated primary antibodies and are particularly suited to applications that commonly use direct conjugates, such as flow cytometry (Figure 20). Using the Zenon reagents to directly label a primary antibody can also eliminate the need for a secondary antibody, thereby avoiding the cross-reactivity limitations of secondary detection methods.

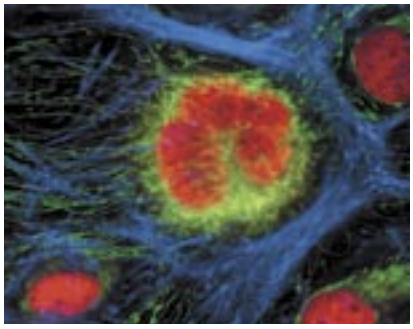


Figure 19. Fixed and permeabilized HeLa cells stained with Alexa Fluor 350 phalloidin and an anti–OxPhos Complex V inhibitor protein antibody to label actin filaments and the mitochondria, respectively. An anti–cdc6 peptide antibody was used to label the nucleus. The anti–OxPhos Complex V inhibitor protein antibody was labeled with the Zenon Alexa Fluor 488 Mouse IgG_1 Labeling Kit, and the anti–cdc6 peptide antibody was labeled with the Zenon Alexa Fluor 568 Mouse IgG_1 Labeling Kit.

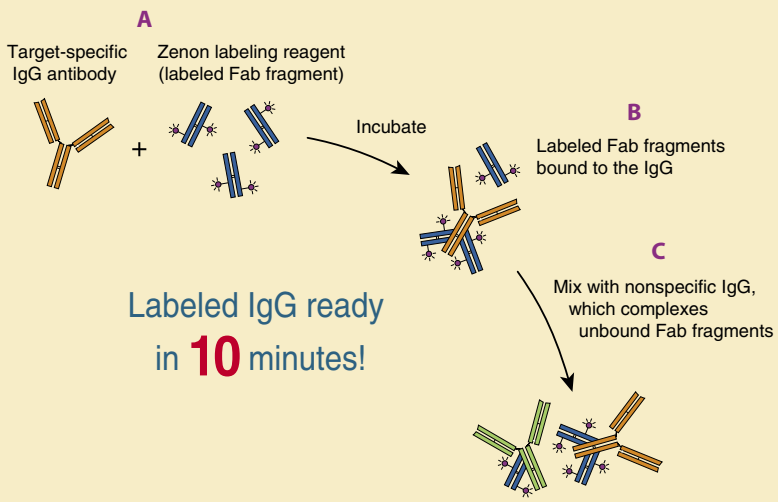


Figure 18. The Zenon labeling scheme. An unlabeled IgG is incubated with the Zenon labeling reagent, which contains a fluorophore-labeled Fab fragment (A). The labeled Fab fragment binds to the Fc portion of the IgG antibody (B), and excess Fab fragment is bound by the addition of a nonspecific IgG (C). The addition of nonspecific IgG prevents cross-labeling of the Fab fragment in experiments where multiple primary antibodies of the same type are present. Note that the Fab fragment used for labeling need not be coupled to a fluorophore, but could instead be coupled to an enzyme or to biotin.

Zenon Labeling Kits (Table 14) are available for labeling mouse IgG_1, IgG_{2a}, and IgG_{2b} antibodies and rabbit IgG antibodies with one of our premier Alexa Fluor dyes, where each kit provides sufficient material for 50 labelings. We also offer **Zenon Tricolor Labeling Kits**, each with a selection of three Zenon labeling reagents for imaging or flow cytometry (Table 15). Information on our Alexa Fluor tandem Zenon labeling reagents can be found on pages 8–9.

For mouse IgG_1 primary antibodies, we have developed the **Zenon Mouse IgG_1 Labeling Kits enhanced with TSA technology**. These enhanced Zenon kits contain components from the Zenon Horseradish Peroxidase Mouse IgG_1 Labeling Kit and the Alexa Fluor 488 or Alexa Fluor 568 TSA Kit. The combined methodology provides the ease of labeling mouse IgG_1 antibodies with Zenon reagents and the signal amplification afforded by the use of the TSA technology. We offer these enhanced Zenon Kits containing either the green-fluorescent Alexa Fluor 488 tyramide or the red-orange–fluorescent Alexa Fluor 568 tyramide.

Table 14. Zenon Alexa Fluor Labeling Kits.

Dye	Mouse IgG_1	Mouse IgG_{2a}	Mouse IgG_{2b}	Rabbit IgG	Goat	Human
Alexa Fluor 350	Z25000	Z25100	Z25200	Z25300		Z25400
Alexa Fluor 405	Z25013	Z25113	Z25213	Z25313		
Alexa Fluor 430	Z25001			Z25301		
Alexa Fluor 488	Z25002	Z25102	Z25202	Z25302	Z25602	Z25402
Alexa Fluor 532	Z25003					
Alexa Fluor 546	Z25004	Z25104	Z25204	Z25304		
Alexa Fluor 555	Z25005	Z25105	Z25205	Z25305	Z25605	Z25405
Alexa Fluor 568	Z25006	Z25106	Z25206	Z25306	Z25606	
Alexa Fluor 594	Z25007	Z25107	Z25207	Z25307	Z25607	Z25407
Alexa Fluor 647	Z25008	Z25108	Z25208	Z25308	Z25608	Z25408
Alexa Fluor 660	Z25009					
Alexa Fluor 680	Z25010	Z25110	Z25210	Z25310		
Alexa Fluor 700	Z25011			Z25311		
Alexa Fluor 750				Z25312		

Table 15. Zenon Tricolor Mouse and Rabbit Labeling Kits.

Zenon Tricolor Kit	Dye	Mouse IgG_1	Mouse IgG_{2a}	Mouse IgG_{2b}	Rabbit
Kit #1 for imaging	Alexa Fluor 488 Alexa Fluor 555 Alexa Fluor 647	Z25060	Z25160	Z25260	Z25360
Kit #2 for imaging	Alexa Fluor 350 Alexa Fluor 488 Alexa Fluor 594	Z25070	Z25170	Z25270	Z25370
Kit #3 for flow cytometry (488 nm excitation)	Alexa Fluor 488 R-phycoerythrin (R-PE) Alexa Fluor 647–R-PE	Z25080	Z25180	Z25280	

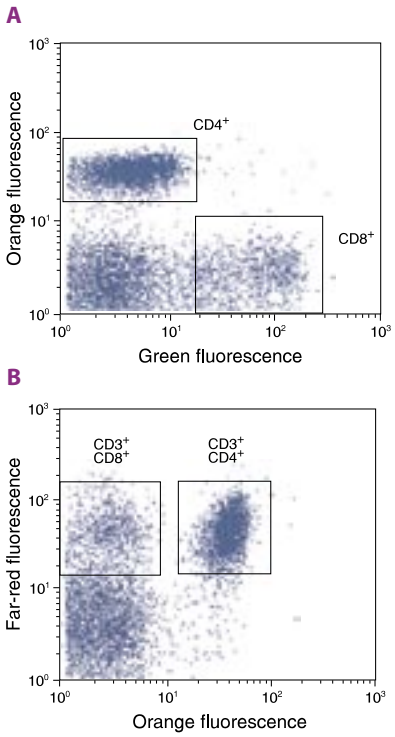


Figure 20. Human peripheral blood lymphocytes were stained with three antibodies: an anti-CD3 mouse IgG_1 antibody prelabeled with the Zenon Alexa Fluor 647 Mouse IgG_1 Labeling Kit, an anti-CD4 mouse IgG_1 antibody prelabeled with the Zenon R-Phycoerythrin Mouse IgG_1 Labeling Kit, and an anti-CD8 mouse IgG_{2a} antibody prelabeled with the Zenon Alexa Fluor 488 Mouse IgG_1 Labeling Kit. Samples were analyzed by flow cytometry. Panels A and B show that cells can be separated by plotting the orange-fluorescent versus green-fluorescent signal or red-fluorescent versus orange-fluorescent signal, respectively, demonstrating that the Zenon label does not transfer to other antibodies in the same sample.

The Zenon Technology Zone. A special location at our website (www.probes.com/zenon) provides direct access to more information about our Zenon labeling technology and its applications, as well as upcoming Zenon product releases. An email address (Zenon@probes.com) is also available for answering questions about Zenon technology and products.

Alexa Fluor Kits for Signal Amplification

Tyramide Signal Amplification with Alexa Fluor Dyes

Tyramide signal amplification (TSA) technology—sometimes called CARD, for catalyzed reporter deposition—is an enzyme-mediated detection method that utilizes the catalytic activity of horseradish peroxidase (HRP) to generate high-density labeling of a target protein or nucleic acid sequence *in situ* (Figure 21). The TSA method has been reported to increase the sensitivity by up to 100-fold compared with conventional avidin–biotinylated enzyme complex (ABC) procedures. The signal amplification conferred by the turnover of multiple tyramide substrates per peroxidase label translates into practical benefits, namely ultrasensitive detection of low-abundance targets in fluorescence *in situ* hybridization, immunohistochemistry, neuroanatomical tracing, and other applications (Figures 22–24). Molecular Probes is pleased to provide **TSA Kits** that use our Alexa Fluor dyes to achieve the ultimate in high-resolution signal amplification technology in cell, tissue, and chromosome applications (Table 16).

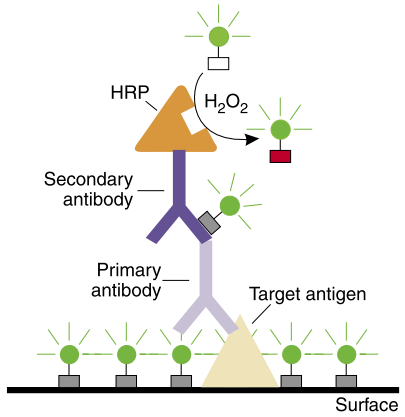


Figure 21. Schematic representation of the TSA detection method applied to immunolabeling of an antigen.

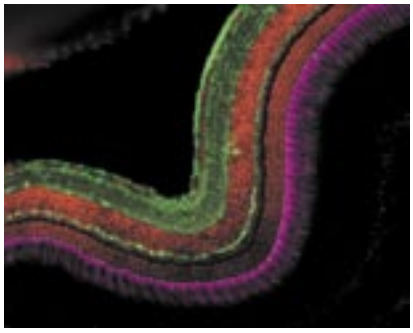


Figure 22. A zebrafish cryosection incubated with the biotin-XX conjugate of mouse monoclonal anti–α-tubulin antibody. The signal was amplified with TSA Kit #22, which includes HRP–streptavidin and Alexa Fluor 488 tyramide. The sample was then incubated with the mouse monoclonal FRet 6 antibody and was visualized with Alexa Fluor 647 goat anti–mouse IgG, which is pseudocolored magenta. Finally, the nuclei were counterstained with SYTOX Orange nucleic acid stain.

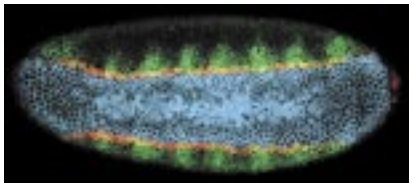


Figure 23. Simultaneous detection of three gene targets in a whole mount *Drosophila* embryo by fluorescence *in situ* hybridization. Pseudocolored green-fluorescent labeling represents a fluorescein-labeled cRNA probe detected using a rabbit anti-fluorescein/Oregon Green primary antibody and an Alexa Fluor 488 dye–labeled anti-rabbit secondary antibody. Pseudocolored yellow- and red-fluorescent labeling represents a biotinylated cRNA probe detected using HRP–streptavidin and Alexa Fluor 568 tyramide (TSA Kit #24). Pseudocolored blue-fluorescent labeling represents a digoxigenin-labeled cRNA probe detected using a mouse anti-digoxigenin primary antibody in conjunction with an Alexa Fluor 647 dye–labeled anti-mouse secondary antibody. Image contributed by Ethan Bier and David Kosman, University of California, San Diego.

Figure 24. *In situ* hybridization of α-satellite probes to human chromosomes 1, 15, and 17 detected by tyramide signal amplification. α-Satellite probes to chromosomes 1, 15, and 17 were labeled by nick translation with biotin-11-dUTP, ChromaTide Texas Red-12-dUTP, and ChromaTide Oregon Green 488-5-dUTP, respectively. Following simultaneous hybridization of all three probes, the biotinylated chromosome 1 probe was detected with HRP–streptavidin and Alexa Fluor 546 tyramide (TSA Kit #23). HRP activity from this first TSA detection step was then quenched by treatment with 1% hydrogen peroxide for 30 minutes. The Oregon Green 488 dye–labeled chromosome 17 probe was then detected with anti-fluorescein/Oregon Green antibody followed by HRP-conjugated goat anti–mouse IgG antibody and Alexa Fluor 594 tyramide (TSA Kit #5). HRP activity from this second TSA detection step was then quenched by treatment with 1% hydrogen peroxide for 30 minutes. The Texas Red dye–labeled chromosome 15 probe was then detected with rabbit anti–Texas Red antibody followed by HRP-conjugated goat anti–rabbit IgG antibody and Alexa Fluor 488 tyramide (TSA Kit #12). After counterstaining with Hoechst 33258, the images were acquired using filters appropriate for DAPI, FITC, TRITC, and the Texas Red dye.

Table 16. Alexa Fluor TSA Kits.

Tyramide Label	Peroxidase Conjugate		
	Anti–Mouse IgG*	Anti–Rabbit IgG*	Streptavidin
Alexa Fluor 350	T20917	T20927	T20937
Alexa Fluor 405	T30950	T30951	T30952
Alexa Fluor 488	T20912	T20922	T20932
Alexa Fluor 546	T20913	T20923	T20933
Alexa Fluor 555	T30953	T30954	T30955
Alexa Fluor 568	T20914	T20924	T20934
Alexa Fluor 594	T20915	T20925	T20935
Alexa Fluor 647	T20916	T20926	T20936

* Host = goat.

Amplify Immunofluorescence Signals—Alexa Fluor Signal-Amplification Kits

Molecular Probes Alexa Fluor Signal-Amplification Kits are designed to substantially increase the signals that can be obtained by immunofluorescence techniques. Each kit takes advantage of the superior brightness and photostability of Alexa Fluor antibody conjugates and makes it possible to detect low-abundance targets that are not visible with other labels. The **Alexa Fluor 488 Signal-Amplification Kit for Fluorescein- and Oregon Green Dye–Conjugated Probes** dramatically enhances the fluorescence and photostability of virtually any fluoresceinated probe (Figure 25). The three kits for mouse antibodies (Table 17) can be used to sensitively detect mouse primary antibodies. All of the Alexa Fluor Signal-Amplification Kits contain a detailed protocol and sufficient reagent to stain ~60 samples of cells adhering to coverslips or ~300 samples for analysis by flow cytometry.

Table 17. Alexa Fluor Signal-Amplification Kits for Mouse Antibodies.

Amplification Kit	Kit Components	Catalog Number
Alexa Fluor 488	Alexa Fluor 488 rabbit anti–mouse IgG Alexa Fluor 488 goat anti–rabbit IgG	A11054
Alexa Fluor 568	Alexa Fluor 568 rabbit anti–mouse IgG Alexa Fluor 568 goat anti–rabbit IgG	A11066
Alexa Fluor 594	Alexa Fluor 594 rabbit anti–mouse IgG Alexa Fluor 594 goat anti–rabbit IgG	A11067

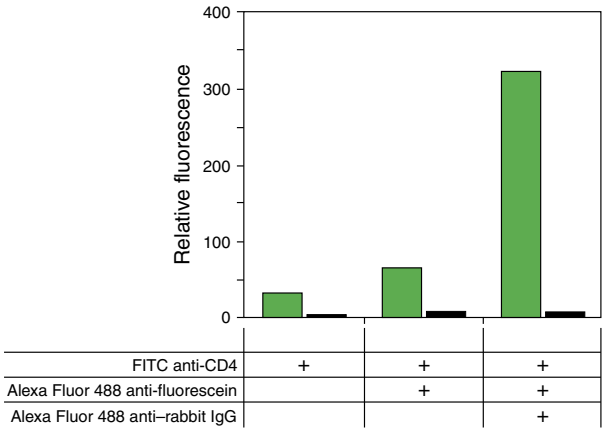


Figure 25. An example of flow cytometry results obtained using the Alexa Fluor 488 Signal-Amplification Kit for Fluorescein- and Oregon Green Dye–Conjugated Probes. Human T-cell leukemia cells (Jurkat) were stained with fluorescein (FITC) mouse anti-CD4 antibody and, as indicated, with Alexa Fluor 488 rabbit anti-fluorescein/Oregon Green antibody and Alexa Fluor 488 goat anti–rabbit IgG antibody. The fluorescence values of the negative controls, in which the FITC anti-CD4 antibody was omitted, are shown (black) together with the fluorescence values of the experimental samples (green). The fluorescence values represent the average signals from the population of cells analyzed.

Tyramide signal amplification combines three elementary processes:

1. Binding of a probe to the target via immunoaffinity (proteins) or hybridization (nucleic acids), and the subsequent binding of an HRP-tagged secondary detection reagent to the probe.
2. HRP-mediated conversion of multiple copies of a fluorescent, biotinylated, or DNP-labeled tyramide derivative to a highly reactive radical.
3. Covalent binding of the reactive, short-lived tyramide radicals to nearby nucleophilic residues, greatly reducing diffusion-related signal loss.

Alexa Fluor Reactive Dyes—Create Your Own Alexa Fluor Conjugate

Amine-Reactive Esters

Our **Alexa Fluor succinimidyl esters** (Table 18) provide the most efficient and easy-to-use reaction chemistry to selectively link an Alexa Fluor dye to accessible primary amine groups on proteins, modified nucleic acids, or other biomolecules. Succinimidyl esters are excellent reagents for amine modification because the covalent bonds they form are as stable as the peptide bonds used to link amino acids in proteins. With these reagents, you can vary both the amount of dye and the target in your labeling reaction to create the perfect Alexa Fluor conjugate for your research application, including FRET (Table 19). These reactive dyes are preferred by Molecular Probes scientists and are also used in our Protein Labeling Kits, Monoclonal Antibody Labeling Kits, Microscale Protein Labeling Kits, and ARES DNA Labeling Kits (pages 17–19; Figure 26).

Additionally, the Alexa Fluor 488 fluorophore is available as a tetrafluorophenyl (TFP) ester. The TFP ester form offers several advantages over the succinimidyl ester form—the TFP ester is more soluble and stable in organic solvents, less susceptible to hydrolysis in aqueous solutions, and more reactive on a per-mole basis.

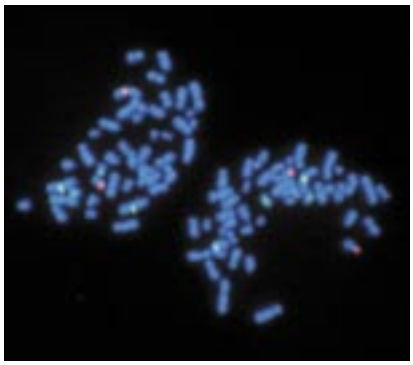


Figure 26. Fluorescent probes generated with ARES DNA Labeling Kits hybridized to human metaphase chromosome spreads. Centromere probes specific for chromosomes 17, 1 and 15 were prepared by nick translation and labeled with kits containing green-fluorescent Alexa Fluor 488, red-orange–fluorescent Alexa Fluor 546 and red-fluorescent Alexa Fluor 594 dyes. DNA was counterstained with the blue-fluorescent Hoechst 33342 dye, and the slides were mounted using the ProLong Antifade Kit. This multiple-exposure image was obtained with bandpass filter sets appropriate for fluorescein, rhodamine, Texas Red dye and DAPI.

Table 18. Alexa Fluor reactive dyes.

Dye	Succinimidyl Ester*	Maleimide	Hg-Link Phenylmercury	Hydrazide	Hydroxyl-amine	Cadaverine
Alexa Fluor 350	A10168	A30505	H30460	A10439	A30627	A30674
Alexa Fluor 405	A30000, A30100	A30458	H30461			A30675
Alexa Fluor 430	A10169					
Alexa Fluor 488	A20000, A20100	A10254	H30462	A10436	A30629	A30676
Alexa Fluor 514	A30002					
Alexa Fluor 532	A20001	A10255				
Alexa Fluor 546	A20002, A20102	A10258				
Alexa Fluor 555	A20009, A20109	A20346	H30463	A20501MP		A30677
Alexa Fluor 568	A20003, A20103	A20341		A10437		A30680
Alexa Fluor 594	A20004, A20104	A10256	H30464	A10438		A30678
Alexa Fluor 610	A30003, A30103					
Alexa Fluor 633	A20005, A20105	A20342		A30634		
Alexa Fluor 647	A20006, A20106	A20347	H30465	A20502	A30632	A30679
Alexa Fluor 660	A20007	A20343				
Alexa Fluor 680	A20008, A20108	A20344				
Alexa Fluor 700	A20010, A20110					
Alexa Fluor 750	A20011, A20111					

* With the exception of the Alexa Fluor 350, Alexa Fluor 405, and Alexa Fluor 430 dyes, which are packaged only in units of 5 mg, the Alexa Fluor succinimidyl esters are packaged in units of either 1 mg or 5 mg.

Table 19. R_0 values for Alexa Fluor dyes.*

Donor	Acceptor					
	Alexa Fluor 488	Alexa Fluor 546	Alexa Fluor 555	Alexa Fluor 568	Alexa Fluor 594	Alexa Fluor 647
Alexa Fluor 350	50					
Alexa Fluor 488	NA	64	70	62	60	56
Alexa Fluor 546		NA		70	71	74
Alexa Fluor 555			NA		47	51
Alexa Fluor 568				NA		82
Alexa Fluor 594					NA	85
Alexa Fluor 647						NA

* R_0 values in angstroms (Å) represent the distance at which fluorescence resonance energy transfer (FRET) from the donor dye to the acceptor dye is 50% efficient. Values were calculated from spectroscopic data. NA = Not applicable.

Maleimides and Hg-Link Reagents for Modification of Thiols

Thiol-reactive dyes are principally used to prepare fluorescent peptides, proteins, and oligonucleotides for probing biological structure, function, and interactions. Because the thiol functional group is not very common in most proteins, thiol-reactive reagents often provide a means of selectively modifying a protein at a defined site. Molecular Probes offers a variety of **Alexa Fluor maleimides** (Table 18). Maleimides are easier to use and less photosensitive than iodoacetamides, making them excellent reagents for thiol-selective modification, quantitation, and analysis.

The Hg-Link phenylmercury reagents (Table 18) react with thiols to yield a reversible mercury–thiol bond. This reaction occurs under the same conditions used for maleimide reactions with thiols, but unlike maleimides, Hg-Link dyes can also react with nitrosylated thiols (SNO) via the Saville reaction. Nitrosylation has important biological effects, and although anti-SNO antibodies are employed for SNO detection, Hg-Link Alexa Fluor dyes have potential use for direct SNO detection in gels, cells, and tissues.

Hydrazides, Hydroxylamines, and Cadaverines for Modification of Aldehydes, Ketones, and Carboxylic Acids

Hydrazine derivatives react with ketones to yield relatively stable hydrazones, and with aldehydes to yield hydrazones that are somewhat less stable, though they may be formed faster. Hydroxylamine derivatives (aminooxy compounds) react with aldehydes and ketones to yield oximes. Both hydrazones and oximes can be reduced with sodium borohydride ($NaBH_4$) to further increase the stability of the linkage. Aliphatic amines such as the cadaverines react with aldehydes and ketones to form Schiff bases that, like the hydrazones and oximes, can be reduced with $NaBH_4$ or sodium cyanoborohydride to form stable secondary amines. Hydroxylamines react better with aldehydes and ketones at neutral pH than do other primary amine–containing reagents such as hydrazine and cadaverine derivatives. Cadaverines, hydroxylamines, and hydrazines can all be coupled to water-soluble carbodiimide–activated carboxylic acid groups in drugs, peptides, and proteins. Hydrazides are also excellent probes for intracellular labeling and neuronal tracing (page 22).

Label Your Own Protein with an Alexa Fluor Dye

Alexa Fluor Protein Labeling Kits

We are pleased to offer protein labeling kits that combine years of protein labeling experience with our premium fluorophores. If you have a protein that you want to label with an Alexa Fluor dye, our kits make it easy:

- The protocols are simple and easy to follow.
- All buffers and purification materials are included.
- The reactive dye is premeasured, so there is no need to weigh out small quantities of dye.
- The procedure (including purification) takes about two hours, with little hands-on time.

Protein Labeling Kits

Our easy-to-use **Protein Labeling Kits** (Table 20, next page) provide a nearly effortless way to label proteins, especially IgG antibodies, with a fluorescent dye. Simply add ~1 mg of protein (in a volume of ~500 µl and free of amine-containing buffers such as Tris) to one of the three included vials, which contain a premeasured quantity of amine-reactive dye and a magnetic stir bar. Purification is accomplished on a gravity-feed size exclusion column, which is supplied with the kit (Figure 27, next page). Although optimized for IgG antibodies, the kits can be used to make conjugates of other proteins larger than ~40,000 daltons.

Monoclonal Antibody Labeling Kits

Molecular Probes **Monoclonal Antibody Labeling Kits** (Table 20) provide researchers with a simple yet efficient means to label small amounts of IgG antibodies with our superior Alexa Fluor dyes. Unlike polyclonal antibodies and most other commercially available proteins, monoclonal antibodies are typically available only in small quantities. Simply adjust the protein concentration to ~1 mg/ml in the provided buffer, then add it to one of the five vials of amine-reactive dye. Purification is accomplished on a size exclusion spin column optimized for proteins of molecular weight ≥40,000 daltons (Figure 28).

Microscale Protein Labeling Kits

The **Microscale Protein Labeling Kits** provide a convenient means for labeling small amounts (20–100 µg) of purified protein with an Alexa Fluor dye. These kits have been optimized for labeling proteins with molecular weights between 12 and 150 kDa, and contain everything needed to perform labeling reactions and to separate the resulting conjugates from excess dye label. Convenient spin columns are used to purify the labeled protein with yields between 60 and 90%, depending primarily on the molecular weight of the starting material. Labeling and purification can be completed in as little as 30 minutes.

Table 20. Alexa Fluor Protein and Monoclonal Antibody Labeling Kits.

Dye	Protein Labeling Kit	Monoclonal Antibody Labeling Kit	Microscale Protein Labeling Kit
Alexa Fluor 350	A10170	A20180	
Alexa Fluor 430	A10171		
Alexa Fluor 488	A10235	A20181	A30006
Alexa Fluor 532	A10236	A20182	
Alexa Fluor 546	A10237	A20183	
Alexa Fluor 555	A20174	A20187	A30007
Alexa Fluor 568	A10238	A20184	
Alexa Fluor 594	A10239	A20185	A30008
Alexa Fluor 633	A20170		
Alexa Fluor 647	A20173	A20186	A30009
Alexa Fluor 660	A20171		
Alexa Fluor 680	A20172		

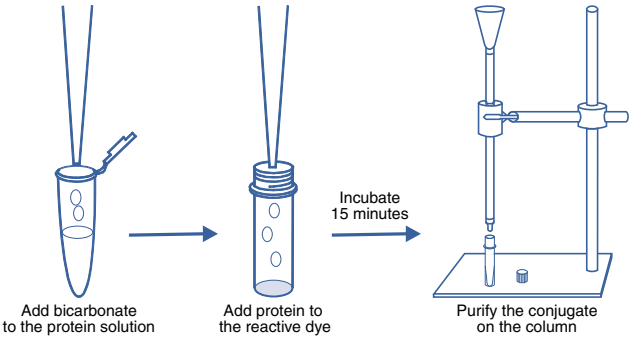


Figure 27. Molecular Probes' Protein Labeling Kits are the simplest way to label proteins.

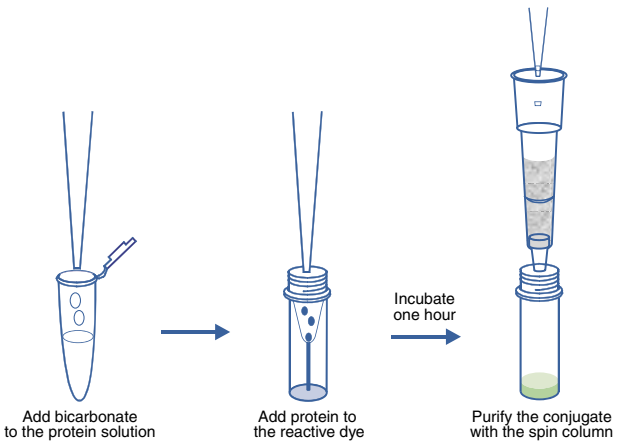


Figure 28. Molecular Probes' Monoclonal Antibody Labeling Kits are the simplest way to label small amounts of IgG antibodies.

Zenon Labeling Technology for IgG Antibodies

As an alternative to direct conjugation of primary antibodies with our Alexa Fluor reactive dyes, we recommend using our exclusive Zenon technology (page 12) to form labeled complexes of mouse IgG_1, IgG_{2a}, and IgG_{2b} and rabbit IgG antibodies. Antibody labeling can be completed in minutes and requires only submicrogram amounts of the antibody; the conjugate brightness can be easily adjusted by modifying the ratio of the reagents.

Alexa Fluor Nucleic Acid Labeling Reagents for Molecular Biology Applications

The spectral diversity of the Alexa Fluor dyes make them ideal tools for multicolor applications such as fluorescence *in situ* hybridization (FISH) and microarray experiments. Our wide selection of dyes spans the visible spectrum and beyond, providing enormous flexibility in choosing a label that is compatible with microarray scanners and fluorescence microscopes. The Alexa Fluor dyes have several properties that make them superior to other fluorescent dyes:

- **High water solubility.** The Alexa Fluor dyes are highly water soluble, making them ideal for hybridization experiments. Nucleic acids labeled with the Alexa Fluor dyes do not aggregate or precipitate, even in high-salt conditions.
- **pH independence.** Fluorescence of the Alexa Fluor conjugates is not pH sensitive in the ranges used for hybridization solutions and microscopy mounting media.
- **Resistance to photobleaching.** The enhanced photostability of Alexa Fluor dyes makes them ideal for applications requiring imaging, such as FISH (Figure 29) and microarrays (Figure 30).
- **High signal correlation.** The Alexa Fluor 555/Alexa Fluor 647 dye pair provides better signal correlation and therefore higher resolution of differentially expressed genes than the commonly used Cy3/Cy5 dye pair (Figure 31).

The products described below offer several different strategies for labeling your samples with our state-of-the-art fluorescent dyes.

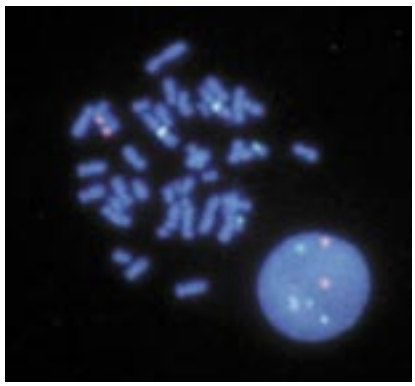


Figure 29. Centromere probes to chromosome 1, chromosome 15, and chromosome 17 were labeled with the ULYSIS Alexa Fluor 546, Alexa Fluor 594, and Oregon Green 488 Nucleic Acid Labeling Kits, respectively, and hybridized to human metaphase chromosomes. The chromosomes were then counterstained with Hoechst 33342.

ARES DNA Labeling Kits

Nucleic acids labeled with our **ARES DNA Labeling Kits** (Table 21, next page) are ideal for FISH or microarray experiments. The kits provide a versatile, two-step method for labeling DNA with fluorescent dyes (Figure 32). In the first step, an aminoallyl dUTP is enzymatically incorporated into DNA. In the second step, a succinimidyl ester dye reacts with the amine-modified DNA to form a permanent covalent bond. This method achieves a very high and consistent labeling efficiency, regardless of the dye you choose. The labeling protocols provided generally result in about one dye per 12–20 bases, which we have determined to be optimal for FISH and microarray hybridization.

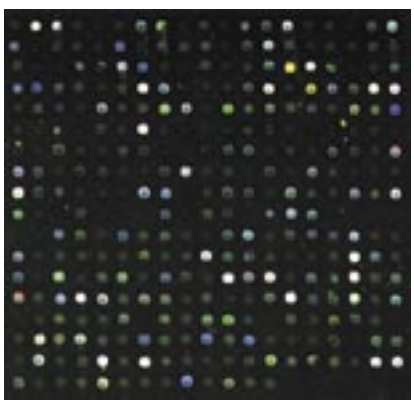


Figure 30. A cDNA microarray hybridized with three different cDNA samples. Each sample was labeled with a different dye using the ARES DNA Labeling Kits, and then imaged separately using a PerkinElmer ScanArray 5000 microarray scanner. The black-and-white images were pseudocolored so that blue represents the Alexa Fluor 647 dye, green represents the Alexa Fluor 488 dye, and red represents the Alexa Fluor 594 dye. The images were overlaid using an additive RGB color scheme to reveal spots that show differential expression. Where fluorescence from all colors is equivalent, spots are white; where blue is low, spots are yellow; where green is low, spots are pink; and where red is low, spots are aqua. Image provided by Gerti Schut and Mike Adams, University of Georgia.

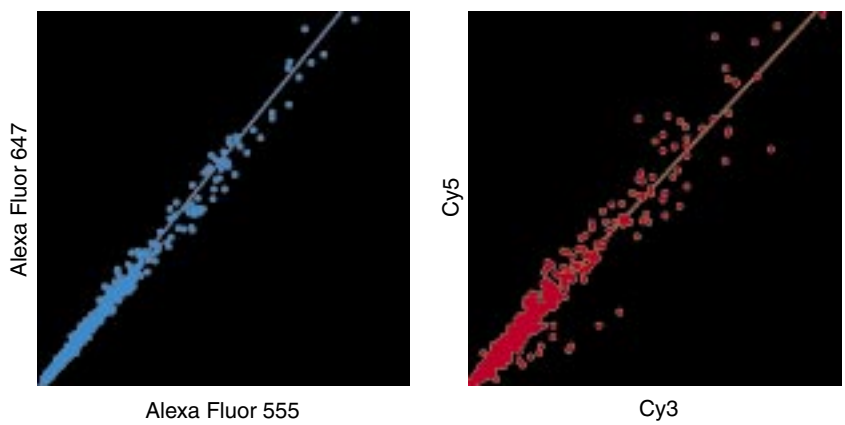


Figure 31. Hybridization signals of the Alexa Fluor 555/647 dye pair show a higher degree of signal coincidence than the Cy3/Cy5 dye pair.

ULYSIS Nucleic Acid Labeling Kits

ULYSIS Nucleic Acid Labeling Kits (Table 21) use the versatile, patented Universal Linkage System (ULS) chemistry developed at KREATECH Diagnostics to label DNA or RNA with the Alexa Fluor dyes. The ULS labeling technique directly labels nucleic acids on the *N*-7 position of guanine without the need for enzymatic incorporation of modified nucleotides. The labeling reaction requires only 15 minutes, and separation of the labeled nucleic acids from the unreacted ULS complex can be accomplished through the use of a simple spin-column procedure (Figure 33). DNA longer than ~1,000 base pairs requires a 10 minute DNase digestion before labeling, which both optimizes labeling and fragments the probe for efficient hybridization. Nucleic acids labeled using the ULYSIS kits are stable indefinitely and hybridize effectively to target DNA. The ULS method has been used to prepare labeled probes for dot, Southern and Northern blot analysis, RNA and DNA *in situ* hybridization, multicolor fluorescence *in situ* hybridization (FISH), comparative genome hybridization (CGH), and microarray analysis.

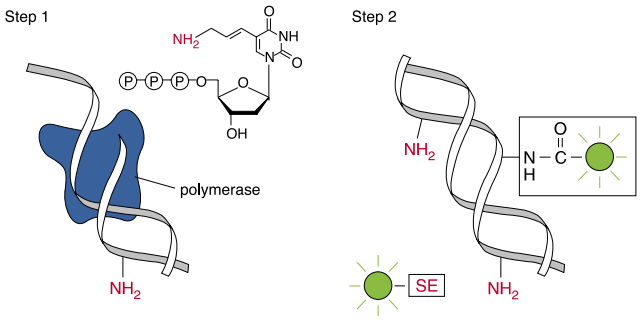


Figure 32. Schematic diagram of the labeling method provided in our ARES DNA Labeling Kits. The ARES DNA Labeling Kits use a two-step method to label DNA. Step 1) The aminoallyl dUTP is enzymatically incorporated. Step 2) A reactive fluorophore is used to label the incorporated aminoallyl group.

Table 21. Alexa Fluor Kits for labeling nucleic acids.

Dye	ULYSIS Nucleic Acid Labeling Kit	ARES DNA Labeling Kit	Oligonucleotide Amine Labeling Kit
Alexa Fluor 488	U21650	A21665	A20191
Alexa Fluor 532	U21651	A21666	
Alexa Fluor 546	U21652	A21667	
Alexa Fluor 555		A21677	
Alexa Fluor 568	U21653	A21668	
Alexa Fluor 594	U21654	A21669	
Alexa Fluor 647	U21660	A21676	A20196
Alexa Fluor 660	U21656		

Alexa Fluor Nucleotides for Labeling Nucleic Acids

Molecular Probes offers a series of **uridine triphosphates** (UTP) and **deoxyuridine** or **deoxycytidine** triphosphates (dUTP, OBEA-dCTP) conjugated to our superior Alexa Fluor dyes (Table 22). The ChromaTide nucleotides are useful for generating labeled nucleic acids for molecular biology and molecular cytogenetics applications, including chromosome and mRNA FISH experiments; gene expression studies and mutation detection on arrays and microarrays; and *in situ* PCR and RT-PCR. The 5-aminohexylacrylamido-dUTP (aha-dUTP) nucleotides are modified at the *C*-5 position of uridine with a unique hexylacrylamide linker, which serves as a spacer between the nucleotide and the dye. The spacer reduces interactions between the nucleotide and the dye, yielding brighter conjugates. Our extensive selection of fluorescent labels provides the ideal tools for multicolor techniques such as spectral karyotyping, multilocus FISH analysis, "chromosome painting," and comparative genome hybridization.

Table 22. Alexa Fluor ChromaTide nucleotides and aha-dUTP.

Dye	ChromaTide dUTP	ChromaTide OBEA-dCTP	ChromaTide UTP	aha-dUTP
Alexa Fluor 488	C11397	C21555	C11403	
Alexa Fluor 532	C11398			
Alexa Fluor 546	C11401	C21556	C11404	
Alexa Fluor 555				A32762
Alexa Fluor 568	C11399			
Alexa Fluor 647		C21559		A32763

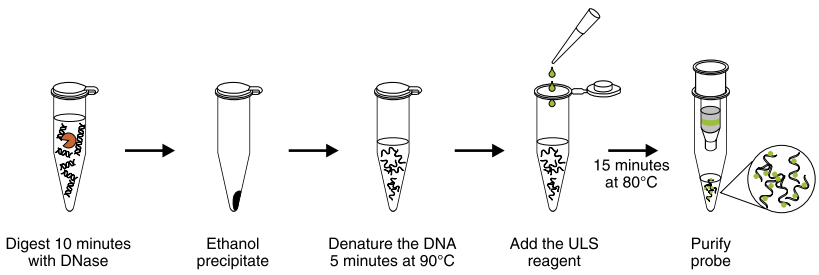


Figure 33. The nucleic acid labeling method provided in our ULYSIS Nucleic Acid Labeling Kits.

Alexa Fluor Oligonucleotide Amine Labeling Kits

The **Alexa Fluor Oligonucleotide Amine Labeling Kits** (Table 18, page 16) provide the reagents required for labeling synthetic oligonucleotides that have amine groups at their 5′-termini. Following purification by standard chromatographic or electrophoretic procedures, these singly labeled oligonucleotides can serve as primers for a variety of applications. The dye-labeled oligonucleotides may also serve as either fluorescence resonance energy transfer (FRET) acceptors or donors in hybridization reactions.

Controls for Microarrays

The techniques available for creating arrays of nucleic acids on solid supports vary widely in reproducibility. Variability in the amount of nucleic acid in each spot on the array can lead to artifactual differences in signal. Fluorescently labeled random-sequence oligonucleotides provide a method for assessing the level of nucleic acids immobilized on solid supports. This method assays the capability of spotted DNA to hybridize, making it possible to determine if hybridization efficiency varies across the array. Our **Panomer 9 random oligodeoxynucleotides** (Table 23) are ideal for this application (Figure 34). These 9-base, random-sequence oligodeoxynucleotides are covalently labeled on the 5′-ends with an Alexa Fluor dye. The Alexa Fluor dye series allows you to use any fluorescence channel of interest and to compare relative signal intensities per spot in several different channels. It is also possible to use the Panomer 9 oligodeoxynucleotides for quality control of spotting techniques or to assay the stability of DNA spots after the array is subjected to washing, boiling, hybridization, or other conditions.

PARAGON DNA Microarray QC Hybridization Kits #1 and #2 (P32934, P32937) consist of a Panomer 9 oligodeoxynucleotide labeled with Alexa Fluor 555 dye or Alexa Fluor 647 dye, respectively, a control microarray slide, and reagents for performing a test hybridization. **PARAGON Genomic DNA Hybridization Test Kits #1 and #2** (P32938, P32942) include human male genomic DNA labeled with Alexa Fluor 555 dye or Alexa Fluor 647 dye, respectively. These **Alexa Fluor 555** and **Alexa Fluor 647 labeled genomic DNAs** are also available separately.

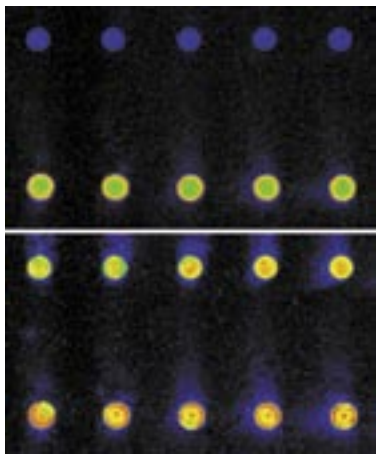


Figure 34. Use of Panomer 9 Alexa Fluor 546 random oligonucleotides to examine microarray spot morphology. Double-stranded calf thymus DNA was spotted out of either a 3X SSC solution (top rows) or 50% DMSO (bottom rows). Panel A shows the microarray spots after hybridization with Panomer 9 Alexa Fluor 546 random oligodeoxynucleotides; DNA spotted out of 3X SSC is much less available for hybridization than is DNA spotted out of 50% DMSO. Panel B shows the same spots after heating, rehybridizing with Panomer 9 Alexa Fluor 546 oligonucleotides, and imaging under the same conditions. The images are pseudocolored so that the series white-red-yellow-green-blue represents decreasing intensity.

Alexa Fluor Reactive Dye Decapacks for Microarray Experiments

For labeling amine-modified DNA or RNA probes for microarray experiments, we offer four of our amine-reactive Alexa Fluor dyes that best match the spectral properties of array scanners, in convenient packages of 10 single-use vials (Table 24). A set containing both the **Alexa Fluor 555** and **Alexa Fluor 647 reactive dye decapacks** (A32755) is also available for two-color experiments. These reactive dyes are rigorously tested for the ability to efficiently label aminoallyl-modified DNA, and can be used in conjunction with our **aminohexylacrylamido-dUTP** (aha-dUTP), **aminoallyl dUTP** or **aminoallyl UTP**) nucleotides, or commercially available aminoallyl nucleotide–based nucleic acid labeling kits.

Table 23. Alexa Fluor Panomer 9 random oligodeoxynucleotides.

Dye	Catalog Number
Alexa Fluor 488	P21680
Alexa Fluor 546	P21681
Alexa Fluor 555	P21687
Alexa Fluor 594	P21682
Alexa Fluor 647	P21686
Alexa Fluor 660	P21684

Table 24. Alexa Fluor reactive dye decapacks.

Dye	Catalog Number
Alexa Fluor 488	A32750
Alexa Fluor 555	A32756
Alexa Fluor 594	A32751
Alexa Fluor 647	A32757

Alexa Fluor Probes for Neuroscience

Visualize Fine Structures or Study Gap Junctions

Alexa Fluor hydrazides (Table 18, page 16) are small polar tracers with high water solubility (typically greater than 8% w/v) and a hydrazide reactive group to permit their fixation by commonly used aldehyde-based fixatives. With their bright, photostable fluorescence and absorption spectra matched to the laser sources of many confocal laser-scanning microscopes, these probes should have great utility for visualizing fine neuronal processes or for studying cell–cell communication via gap junctions. Alexa Fluor hydrazides can be loaded into cells either by microinjection or by using our **Influx pinocytic cell-loading reagent** (I14402, Figure 35). Our **polyclonal antibody to the Alexa Fluor 488 fluorophore** (A11094) can be used in conjunction with other reagents to convert the fluorescence signal from Alexa Fluor 488 hydrazide to an electron-dense precipitate that can be visualized by electron microscopy. Our expertly prepared Alexa Fluor labeled secondary antibodies (Tables 2–4, pages 6 and 7) are also beneficial in this application (Figures 36 and 37).

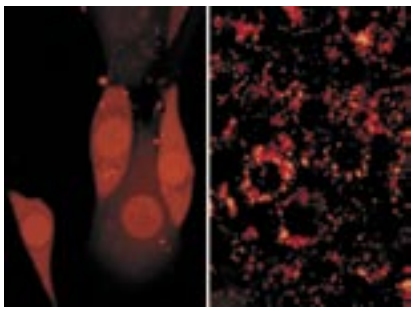


Figure 35. Human cheek epithelial cells labeled with Alexa Fluor 350 wheat germ agglutinin and stained with SYTOX Green nucleic acid stain. This multiple-exposure image was acquired using bandpass filter sets appropriate for DAPI and fluorescein.

Amplify Signals in Dye-Filled Neurons

Alexa Fluor 488 biocytin (A12924), **Alexa Fluor 546 biocytin** (A12923), and **Alexa Fluor 594 biocytin** (A12922) allow you to amplify signals, especially in the finer processes of dye-filled neurons. Alexa Fluor biocytins contain primary amines and can therefore be fixed in cells with formaldehyde or glutaraldehyde. They can then be detected via their biotin moiety using fluorescent- or enzyme-labeled avidin or streptavidin secondary detection reagents (Table 10, page 10).

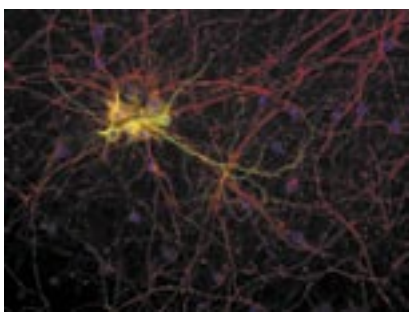


Figure 36. Dendrites from a primary rat neocortical culture were labeled with a mouse monoclonal anti–microtubule associated protein (MAP2) antibody and visualized using green-fluorescent Alexa Fluor 488 goat anti–mouse IgG. Axons were identified with a rabbit anti–neurofilament medium (NF-M, human) antibody that was visualized using red-fluorescent Alexa Fluor 594 goat anti–rabbit IgG. Nuclei were stained with nuclear yellow, which produced fluorescence that was pseudocolored blue. Image contributed by Adele Vincent, University of Tasmania.

Fluorescent Dextrans

Molecular Probes offers fluorescent **dextran conjugates** labeled with our superior Alexa Fluor dyes (Table 25). The Alexa Fluor 488 dye makes the fixable Alexa Fluor 488 dextran conjugate a superior alternative to fluoro-emerald and other green-fluorescent dextran tracers. Fluorescent dextrans also serve as markers for cell loading of macromolecules by microinjection, vesicular fusion, and electroporation, as well as for the uptake and internal processing of exogenous materials by phagocytic cells, long-term tracing of live cells, and endocytic pathways.

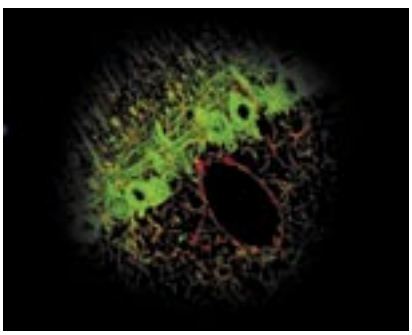


Figure 37. Filamentous structures of neuronal cells in a rat cerebellum, fluorescently labeled to differentiate the cell types. The cerebellum section was probed with primary antibodies to neurofilament and glial fibrillary acidic proteins (GFAP) and subsequently visualized with the green-fluorescent Alexa Fluor 488 goat anti–mouse IgG and red-fluorescent Alexa Fluor 568 goat anti–rabbit IgG antibodies. Image contributed by Gillian Davidson, Andrew Hubbard, and Chris Guerin, Neurotoxicology Group, M.R.C Toxicology Unit, University of Leicester, Leicester, U.K.

Table 25. Alexa Fluor 10,000 MW dextran conjugates.

Dextran Label	Catalog Number
Alexa Fluor 488	D22910
Alexa Fluor 546	D22911
Alexa Fluor 568	D22912
Alexa Fluor 594	D22913
Alexa Fluor 647	D22914

Alexa Fluor Protein Conjugates

Unlike the polydisperse dextrans (Table 25), fluorescent protein tracers have molecular weights that are reasonably well defined (bovine serum albumin (BSA), ~66,000 daltons; codfish parvalbumin 12,328 daltons; ovalbumin ~45,000 daltons; monomeric subunit B of cholera toxin, ~12,000 daltons. These proteins are available as conjugates with various Alexa Fluor dyes (Tables 26 and 35). Some of their applications are similar to those of dextran tracers, although protein conjugates may be more susceptible to proteolysis.

Table 26. Alexa Fluor protein conjugates.

Dye	Protein	Catalog Number
Alexa Fluor 488	Ovalbumin	O34781
Alexa Fluor 555	Ovalbumin	O34782
Alexa Fluor 594	Ovalbumin	O34783
Alexa Fluor 674	Ovalbumin	O34784
Alexa Fluor 488	BSA	A13100
Alexa Fluor 594	BSA	A13101
Alexa Fluor 647	BSA	A34785
Alexa Fluor 488	Codfish parvalbumin	P23012

Alexa Fluor Conjugates for Neurotransmitter Receptors

Because receptor-mediated signal transduction underlies much of what occurs in cellular biochemistry and physiology, fluorescent receptor ligands can provide a sensitive means of identifying and localizing some of the most pivotal molecules in cell biology. We offer Alexa Fluor conjugates of ligands for various cellular receptors, ion channels and ion carriers (Table 27). Many of these site-selective fluorescent probes may be used on live or fixed cells, as well as in cell-free extracts.

Table 27. Alexa Fluor conjugates for neurotransmitter receptors.

Receptor	Alexa Fluor Conjugate	Application
Nicotinic acetylcholine receptor (nicotinic AChR)	Alexa Fluor 488 α-bungarotoxin conjugate (B13422)	Visualizing acetylcholine (ACh) receptors in skeletal muscle, rat myotubules, transformed *Escherichia coli*, and the electric organ from *Torpedo californica*
	Alexa Fluor 594 α-bungarotoxin conjugate (B13423)	
	Alexa Fluor 647 α-bungarotoxin conjugate (B35450)	
G-protein–coupled receptor subtypes AT1 and AT2	Alexa Fluor 488 conjugate of angiotensin II (A13439)	May be useful for imaging the distribution of these receptors

Table 28. Alexa Fluor phalloidins.

Phalloidin Conjugate	Catalog Number
Alexa Fluor 350	A22281
Alexa Fluor 488	A12379
Alexa Fluor 532	A22282
Alexa Fluor 546	A22283
Alexa Fluor 568	A12380
Alexa Fluor 594	A12381
Alexa Fluor 633	A22284
Alexa Fluor 635	A34054
Alexa Fluor 647	A22287
Alexa Fluor 660	A22285
Alexa Fluor 680	A22286

Alexa Fluor Cytoskeleton Probes

Stain F-Actin Filaments

The **Alexa Fluor phalloidins** (Table 28) are by far the best probes for fluorescently labeling F-actin in fixed cells (Figures 38 and 39, next page) and permeabilized tissue (Figure 40). For example, Alexa Fluor 488 phalloidin exhibits considerably better photostability than does fluorescein phalloidin (Figure 41).

Stain Monomeric G-Actin

Alexa Fluor 488 and **Alexa Fluor 594 conjugates of DNase I** (D12371, D12372) selectively label G-actin. DNase I and phalloidin conjugates are perfect for simultaneous visualization of G-actin pools and F-actin.

Study Cytoskeletal Dynamics

Fluorescently labeled actin is an important tool for investigating cytoskeleton dynamics *in vitro*. The **Alexa Fluor conjugates of rabbit skeletal muscle actin** are available with four different labels (Table 29).

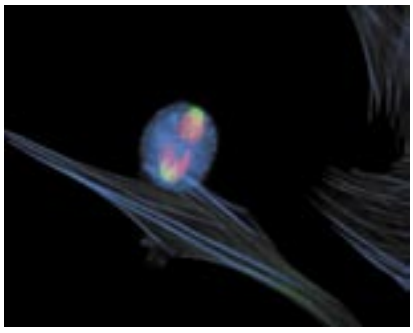


Figure 38. An anaphase muntjac skin fibroblast stained with Alexa Fluor 350 phalloidin, an anti–α-tubulin antibody and an anti–cdc6 peptide antibody. The anti–α-tubulin antibody was prelabeled with the Zenon Alexa Fluor 488 Mouse IgG_1 Labeling Kit, and the anti–cdc6 peptide antibody was prelabeled with the Zenon Alexa Fluor 647 Mouse IgG_1 Labeling Kit.

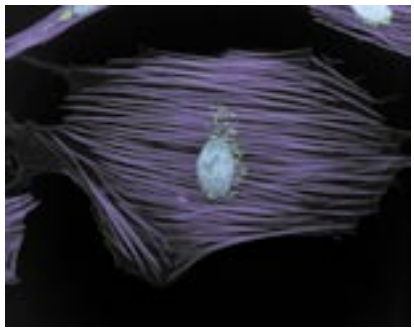


Figure 39. Muntjac skin cells labeled in three colors. Golgi bodies were labeled with anti–golgin-97 antibody and visualized with green-fluorescent Alexa Fluor 488 goat anti–mouse IgG_1 antibody. Filamentous actin (F-actin) was labeled with Alexa Fluor 680 phalloidin (pseudocolored purple). Nuclei were stained with blue-fluorescent DAPI.

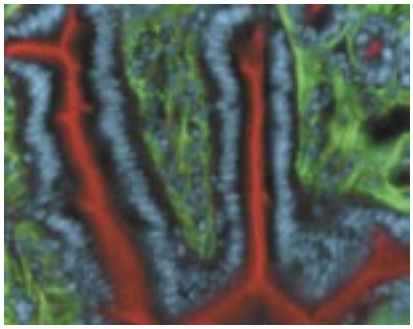


Figure 40. A section of mouse intestine stained with a combination of fluorescent stains. Fibronectin, an extracellular matrix adhesion molecule, was labeled using a chicken primary antibody against fibronectin and visualized using green-fluorescent Alexa Fluor 488 goat anti–chicken IgG antibody. The filamentous actin (F-actin) prevalent in the brush border was stained with red-fluorescent Alexa Fluor 568 phalloidin. Nuclei were stained with DAPI.

Table 29. Alexa Fluor actin conjugates.

Dye	Catalog Number
Alexa Fluor 488	A12373
Alexa Fluor 568	A12374
Alexa Fluor 594	A34050
Alexa Fluor 647	A34051

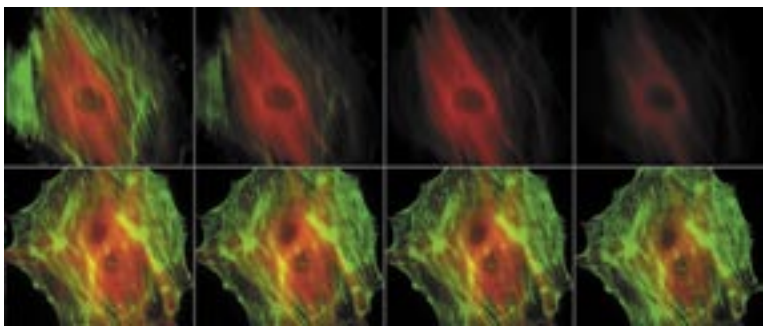


Figure 41. Comparison of the photobleaching rates of the Alexa Fluor 488 and Alexa Fluor 546 dyes and the well-known fluorescein and Cy3 fluorophores. The cytoskeleton of bovine pulmonary artery endothelial cells was labeled with (bottom) Alexa Fluor 488 phalloidin and mouse monoclonal anti–α-tubulin antibody in combination with Alexa Fluor 546 goat anti–mouse IgG antibody or (top) fluorescein phalloidin and the anti–α-tubulin antibody in combination with a commercially available Cy3 goat anti–mouse IgG antibody. The pseudocolored images were taken at 30-second intervals (0, 30, 90, and 210 seconds of exposure). The images were acquired with bandpass filter sets appropriate for fluorescein and rhodamine.

Alexa Fluor Conjugates for Receptor-Mediated Endocytosis and Phagocytosis

Alexa Fluor Transferrin Conjugates

Transferrin is a monomeric serum glycoprotein that binds up to two Fe^{3+} atoms for delivery to vertebrate cells through receptor-mediated endocytosis. In recent research, fluorescently labeled transferrin has been used to measure transferrin-receptor–binding affinity in mammals and parasites, to investigate endocytosis, and to study endocytic recycling pathways (Figure 42). Molecular Probes offers a wide range of **Alexa Fluor conjugates of transferrin from human serum** (Table 30) for these and other research applications.

Alexa Fluor Conjugates of Lipopolysaccharides

Lipopolysaccharides (LPS) or endotoxins are complex macromolecules present on the outer cell walls of gram-negative bacteria. Recognition of LPS by binding to the CD14 cell-surface receptor of phagocytes is the key initiation step in the mammalian immune response to infection by gram-negative bacteria. We currently offer several **Alexa Fluor conjugates of LPS from *Escherichia coli*** and ***Salmonella minnesota*** (Table 31) to follow binding, transport, and cell internalization processes of LPS.

Alexa Fluor Acetylated Low-Density Lipoprotein

If the lysine residues of the apoprotein of low-density lipoprotein (LDL) have been acetylated, the LDL complex no longer binds to the LDL receptor, but is instead taken up by macrophage and endothelial cells that possess "scavenger" receptors specific for the modified LDL. The superior fluorescence output by our **Alexa Fluor 488 AcLDL** (L23380) and **Alexa Fluor 594 AcLDL** (L35353) provides easier identification of macrophages and endothelial cells in mixed cell populations. With spectral properties almost identical to fluorescein, the Alexa Fluor 488 conjugate is ideal for both microscopy and flow cytometry. The Alexa Fluor 594 conjugate provides a bright red fluorescent signal for use in conjunction with GFP or other green fluorophores.

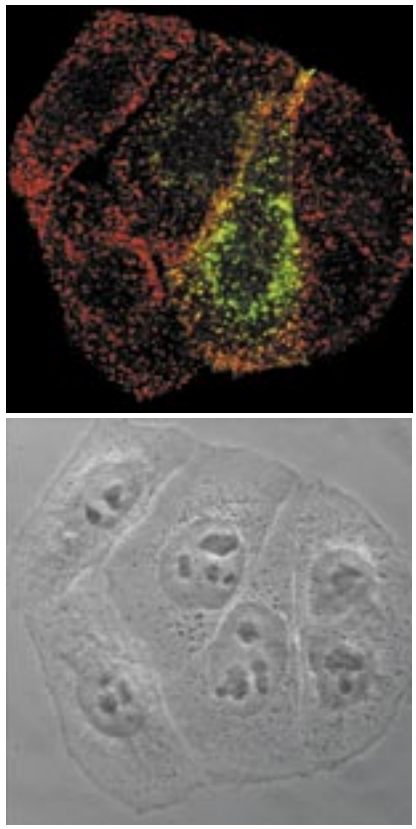


Figure 42. Live HeLa cells incubated with Alexa Fluor 594 transferrin for 10 minutes to label early endosomes. The cells were subsequently fixed with paraformaldehyde and labeled with an antibody to the endosomal protein RhoD; that antibody was visualized with a green-fluorescent secondary antibody. Yellow fluorescence indicates regions of co-localization. To illustrate the staining pattern, the cells were imaged by both fluorescence (top) and differential interference contrast (DIC) microscopy (bottom). Image contributed by Harry Mellor, University of Bristol.

Table 30. Alexa Fluor transferrin conjugates.

Dye	Catalog Number
Alexa Fluor 488	T13342
Alexa Fluor 546	T23364
Alexa Fluor 555	T35352
Alexa Fluor 568	T23365
Alexa Fluor 594	T13343
Alexa Fluor 633	T23362
Alexa Fluor 647	T23366

Table 31. Alexa Fluor lipopolysaccharide conjugates.

Dye	*Escherichia coli*	*Salmonella minnesota*
Alexa Fluor 488	L23351	L23356
Alexa Fluor 568	L23352	
Alexa Fluor 594	L23353	

Fluorescent Epidermal Growth Factor

Fluorescently labeled epidermal growth factor (EGF) can be used to follow lateral mobility and endocytosis of the EGF receptor; however, EGF directly labeled with a fluorophore may not be easily detected. Molecular Probes offers **biotinylated EGF complexed to Alexa Fluor 488 streptavidin** (E13345, Figure 43), **Alexa Fluor 555 streptavidin** (E35350), or **Alexa Fluor 647 streptavidin** (E35351). By attaching an Alexa Fluor dye to the streptavidin (52,800 daltons) instead of to the EGF polypeptide (6,045 daltons), we were able to increase the number of fluorophores per EGF, thus yielding several-fold brighter signals per EGF receptor when compared with conventional conjugates.

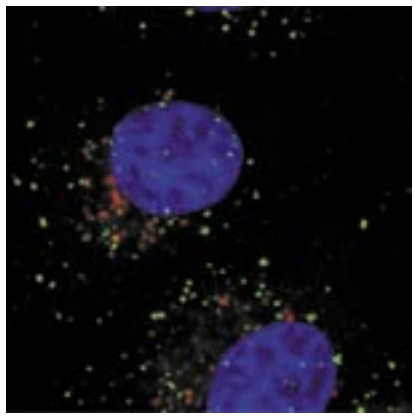


Figure 43. Early endosomes in live HeLa cells identified after a 10 minute incubation with green-fluorescent Alexa Fluor 488 epidermal growth factor. The cells were subsequently fixed with paraformaldehyde and labeled with an antibody to the late endosomal protein, RhoB, then visualized with a red-orange–fluorescent secondary antibody. Nuclei were stained with TO-PRO-3 iodide (pseudocolored blue). Image contributed by Harry Mellor, University of Bristol.

Fluorescent Bacteria and Yeast for Monitoring Phagocytosis

Fluorescent bacteria and yeast particles are proven tools for studying a variety of parameters influencing phagocytosis. Molecular Probes offers green- and red-fluorescent Alexa Fluor conjugates of our BioParticles products (Table 32), a series of fluorescently labeled, heat- or chemical-killed bacteria and yeast.

Table 32. Alexa Fluor BioParticles products.

BioParticles Product	Alexa Fluor 488 Conjugate	Alexa Fluor 594 Conjugate
Escherichia coli (K-12 strain)	E13231	E23370
Staphylococcus aureus (Wood strain without protein A)	S23371	S23372
zymosan A (*Saccharomyces cerevisiae*)	Z23373	Z23374

Alexa Fluor Bioconjugates and Assay Kits for Apoptosis and Cell Proliferation

Annexin V Conjugates for Apoptosis Detection

In normal viable cells, phosphatidyl serine (PS) is located on the cytoplasmic surface of the cell membrane. In apoptotic cells, however, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment. Annexin V has a high affinity for PS and therefore can be used as an effective marker for apoptosis by binding to PS exposed on the cell surface (Figure 44). In collaboration with NeXins Research BV, we offer recombinant **annexin V** conjugated to a wide range of our excellent Alexa Fluor dyes (Table 33).

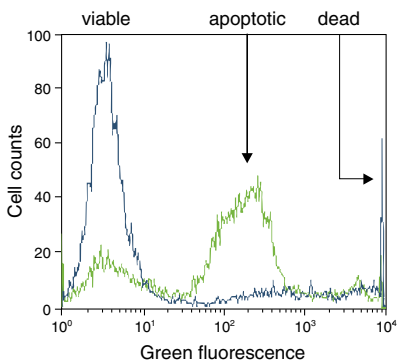


Figure 44. Jurkat human T-cell leukemia cells treated with 10 µM camptothecin for four hours (black line) or untreated (as control, blue line). Cells were then treated with the reagents in the Vybrant Apoptosis Assay Kit #1, followed by flow cytometric analysis. Note that the camptothecin-treated cells (green line) have a significantly higher percentage of apoptotic cells (intermediate green fluorescence) than the basal level of apoptosis seen in the control cells (blue line).

APO-BrdU TUNEL Assay Kit

Because DNA fragmentation is one of the most reliable methods for detecting apoptosis, we have collaborated with Phoenix Flow Systems to offer the **APO-BrdU TUNEL Assay Kit** (A23210), which provides all the materials necessary to label and detect the DNA strand breaks of apoptotic cells. When DNA strands are cleaved or nicked by nucleases, a large number of 3′-hydroxyl ends are exposed. In the APO-BrdU assay, these ends are labeled with BrdUTP and terminal deoxynucleotidyl transferase (TdT) using the terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) technique. Once incorporated into the DNA, BrdU is detected using an Alexa Fluor 488 dye–labeled anti-BrdU monoclonal antibody. This kit also provides propidium iodide for determining total cellular DNA content as well as fixed control cells for assessing assay performance (Figure 45). The Alexa Fluor 488 anti-BrdU antibody is available separately in addition to several other **Alexa Fluor anti-BrdU conjugates** (Table 34).

SelectFX Alexa Fluor 488 Cytochrome *c* Apoptosis Detection Kit

The **SelectFX Alexa Fluor 488 Cytochrome *c* Apoptosis Detection Kit** (S35115) provides all the reagents you need to detect cytochrome *c* in fixed cells. The kit employs an anti–cytochrome *c* primary antibody and an Alexa Fluor 488 dye–labeled secondary antibody. The kit also includes cell fixative and permeabilization reagents, as well as protocols for mammalian cell preparation and staining.

ABSOLUTE-S SBIP Cell Proliferation Assay Kit

The **ABSOLUTE-S SBIP Cell Proliferation Assay Kit** (A23150) uses the SBIP (strand breaks induced by photolysis) methodology, which better preserves cellular features and antigen sites for use in later multiparameter experiments. In the ABSOLUTE-S assay, cells are first incubated in the presence of BrdU, which is incorporated into cellular DNA during replication. BrdU incorporation results in sensitization to photolysis; UV light is used to induce breaks at these sites. In order to amplify the signal, additional BrdU is added at the break sites using a TUNEL procedure described above. Finally, BrdU is detected using an included Alexa Fluor 488 dye–labeled anti-BrdU antibody. This kit is compatible with both flow cytometry and fluorescence microscopy and contains fixed control cells to assist you in interpreting your results. See Table 34 for additional Alexa Fluor anti-BrdU antibodies.

Table 33. Annexin V conjugates and Vybrant Apoptosis Assay Kits.*

Dye	Recommended Dead-Cell Counterstain †	Catalog Number
Annexin V Conjugates		
Alexa Fluor 350	propidium iodide, SYTOX Green	A23202
Alexa Fluor 488	propidium iodide	A13201
Alexa Fluor 568	SYTOX Green	A13202
Alexa Fluor 594	SYTOX Green	A13203
Alexa Fluor 647	SYTOX Green	A23204
Vybrant Apoptosis Kits ‡		
Alexa Fluor 350 §	SYTOX Green	V23200
Alexa Fluor 488	propidium iodide	V13241
Alexa Fluor 488 **	SYTOX Green	V13241

* Provided under license from NeXins Research BV for use in apoptosis detection. † The stains listed in this column stain only necrotic (dead) cells and are spectrally distinct from the associated Alexa Fluor dye, permitting two-color discrimination of apoptotic and necrotic cells. ‡ Vybrant Kits include the counterstains. § Kit uses biotin-X annexin V and Alexa Fluor 350 streptavidin. ** For use in flow cytometry.

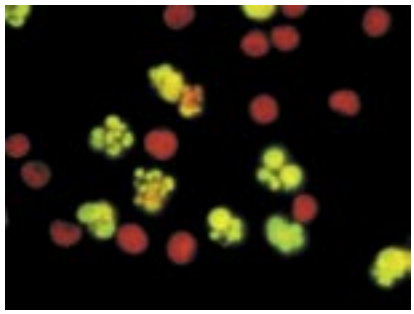


Figure 45. Human lymphoma cells treated with camptothecin for four hours and stained using the APO-BrdU TUNEL Assay Kit. Cells containing DNA strand nicks characteristic of apoptosis are detected by TUNEL and fluoresce green, while necrotic cells are stained with red-fluorescent propidium iodide.

Table 34. Alexa Fluor anti-BrdU conjugates.

Dye	Catalog Number
Alexa Fluor 488	A21303
Alexa Fluor 546	A21308
Alexa Fluor 594	A21304
Alexa Fluor 647	A21305

Kits and Reagents for Labeling Cellular Structures

Kits for Labeling Endoplasmic Reticulum and Peroxisomes

The **SelectFX Alexa Fluor 488 Endoplasmic Reticulum Labeling Kit** (S34200) provides all the reagents required to fix and permeabilize mammalian cells and then label the endoplasmic reticulum (ER). To specifically detect the ER, this kit employs a primary antibody directed against an ER-associated protein, protein disulfide isomerase (PDI), and an Alexa Fluor 488 dye–labeled secondary antibody; fluorescence is observed using standard fluorescein filters.

The **SelectFX Alexa Fluor 488 Peroxisome Labeling Kit** (S34201, Figure 46) provides the reagents required for labeling peroxisomes, including cell fixative and permeabilization reagents. To specifically detect peroxisomes, this kit uses an antibody directed against peroxisomal membrane protein 70 (PMP 70), which is a high-abundance integral membrane protein in peroxisomes, and an Alexa Fluor 488 dye–labeled secondary antibody; fluorescence is observed using standard fluorescein filters.

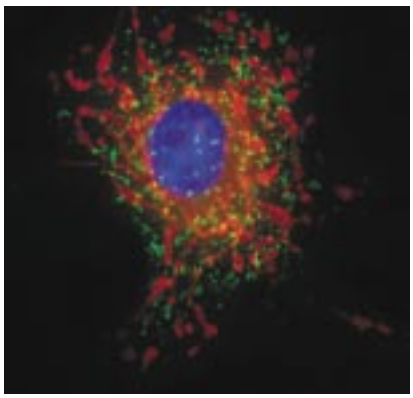


Figure 46. Peroxisome labeling in a fixed and permeabilized bovine pulmonary artery endothelial cell. Peroxisomes were labeled using an antibody directed at peroxisomal membrane protein 70 (PMP-70) and detected with Alexa Fluor 488–labeled goat anti–mouse IgG antibody. Oxidatively stressed mitochondria were stained with MitoTracker Red CMXRos prior to fixation; the nucleus was stained with blue-fluorescent DAPI.

Alexa Fluor Probes for Lipid Rafts

Lipid rafts—membrane microdomains enriched in cholesterol and sphingolipids—segregate specific groups of proteins, thereby providing a hub for cellular signaling and protein trafficking processes. The 12,000-dalton, nontoxic B subunit of cholera toxin (CT-B) binds specifically to the pentasaccharide moiety of ganglioside G_{M1}, a glycosphingolipid predominantly associated with lipid rafts. We offer four **Alexa Fluor dye–labeled CT-B conjugates** (Table 35), which provide a range of spectral characteristics for applications such as colocalization with GFP fusion proteins and fluorescence resonance energy transfer (FRET) imaging.

The **Vybrant Lipid Raft Labeling Kits** (Table 35) provide the key reagents you need to fluorescently label lipid rafts *in situ*. Using the protocol provided, you simply label a live-cell population with an Alexa Fluor conjugate of CT-B; the fluorescent CT-B conjugate binds to the pentasaccharide chain of ganglioside G_{M1}, which selectively partitions into lipid rafts. These CT-B–labeled lipid rafts can then be crosslinked by the anti–CT-B antibody into distinct patches on the plasma membrane, which are more readily visualized by fluorescence microscopy (Figure 47).

Table 35. Alexa Fluor conjugates of cholera toxin subunit B (CT-B) and Vybrant Labeling Kits.

Dye	Conjugate	Vybrant Lipid Raft Labeling Kit
Alexa Fluor 488	C34775	V34403
Alexa Fluor 555	C34776	V34404
Alexa Fluor 594	C34777	V34405
Alexa Fluor 647	C34778	

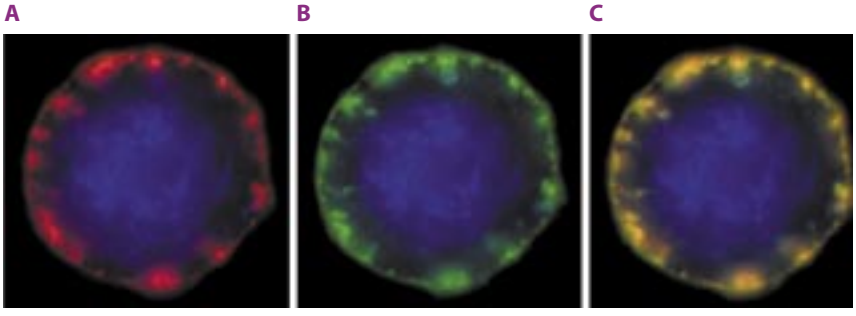


Figure 47. A J774 mouse macrophage cell sequentially stained with BODIPY FL ganglioside G_{M1} and Alexa Fluor 555 dye–labeled cholera toxin subunit B. The cell was then treated with an anti–CT-B antibody to induce CT-B crosslinking. Images of Alexa Fluor 555 dye–labeled cholera toxin subunit B (red fluorescence, Panel A), BODIPY FL ganglioside G_{M1} (green fluorescence, panel B), and Hoechst 33258–stained nuclei (blue fluorescence) were obtained separately using filter sets matched to the dyes' respective spectral characteristics. Images A and B are overlaid in Panel C to emphasize the coincident staining patterns of Alexa Fluor 555 dye–labeled cholera toxin subunit B and BODIPY FL ganglioside G_{M1}.

Alexa Fluor Monoclonal Antibodies Specific for OxPhos Complex IV (Cytochrome Oxidase), Subunit I

Molecular Probes' **Alexa Fluor 488** and **Alexa Fluor 594 conjugates of anti–cytochrome oxidase subunit I** (A21296, A21297) are available for direct staining of mitochondria. These antibodies can be used to visualize mitochondria (Figure 48) or to facilitate the study of cytochrome oxidase (COX) structure and mitochondrial biogenesis.

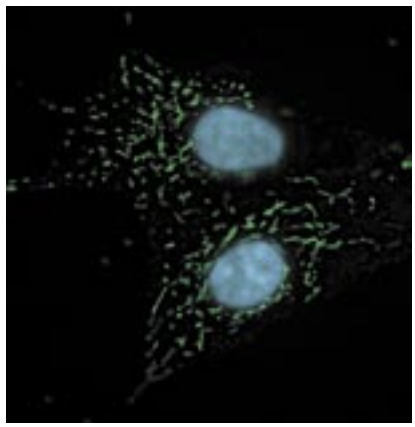


Figure 48. Fixed, permeabilized MRC5 cells were labeled using an Alexa Fluor 488 conjugate of anti–OxPhos Complex IV subunit I antibody and counterstained with DAPI.

Alexa Fluor Prepared Microscope Slides

Molecular Probes' **FluoCells prepared microscope slides** containing cell and tissue preparations stained with our Alexa Fluor conjugates (Table 36). The multicolor staining in these preparations results in stunning, publication-quality images. They are especially useful for setting up microscopes and camera systems and for assessing the capabilities of optical filter sets. When stored properly, these permanently mounted specimens will retain their bright, specific staining patterns for at least 6 months from the date of purchase.

Table 36. Alexa Fluor FluoCells prepared slides.

Product	Contents
FluoCells prepared slide #3 (F24630)	16 µm cryostat section of mouse kidney stained with Alexa Fluor 488 wheat germ agglutinin to label elements of the glomeruli and convoluted tubules (Figure 49). The filamentous actin prevalent in glomeruli and the brush border is stained with Alexa Fluor 568 phalloidin. Nuclei are counterstained with the DNA stain DAPI.
FluoCells prepared slide #4 (F24631)	16 µm cryostat section of mouse intestine stained with Alexa Fluor 350 wheat germ agglutinin to stain the mucus of goblet cells (Figure 50). The filamentous actin prevalent in the brush border is stained with Alexa Fluor 568 phalloidin. Nuclei are stained with SYTOX Green nucleic acid stain.
FluoCells prepared slide #6 (F36925)	Muntjac skin fibroblasts with mitochondria labeled with antibody to OxPhos Complex V inhibitor protein and visualized using orange-fluorescent Alexa Fluor 555 goat anti-mouse IgG (Figure 51). F-actin is labeled with green-fluorescent Alexa Fluor 488 phalloidin. Nuclei are labeled with far red–fluorescent TO-PRO-3 nucleic acid stain.

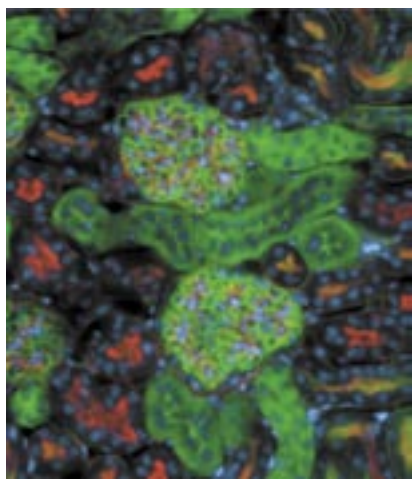


Figure 49. FluoCells prepared slide #3 contains a section of mouse kidney stained with a combination of fluorescent dyes. Alexa Fluor 488 wheat germ agglutinin, a green-fluorescent lectin, was used to label elements of the glomeruli and convoluted tubules. The filamentous actin prevalent in glomeruli and the brush border were stained with red-fluorescent Alexa Fluor 568 phalloidin. Finally, the nuclei were counterstained with the blue-fluorescent DNA stain DAPI. This image is a composite of three micrographs acquired using filter sets appropriate for fluorescein, tetramethylrhodamine, and DAPI.

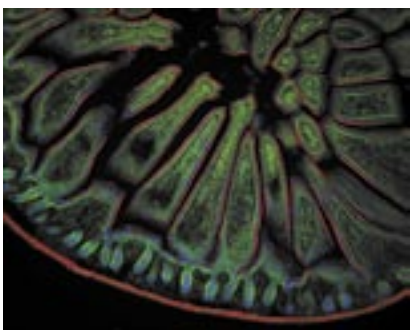


Figure 50. FluoCells prepared slide #4 contains a section of mouse intestine stained with a combination of fluorescent stains. Alexa Fluor 350 wheat germ agglutinin is a blue-fluorescent lectin that was used to stain the mucus of goblet cells. The filamentous actin prevalent in the brush border was stained with red-fluorescent Alexa Fluor 568 phalloidin. Finally, the nuclei were stained with SYTOX Green nucleic acid stain. This image is a composite of three digitized images obtained with filter sets appropriate for fluorescein, DAPI, and tetramethylrhodamine.

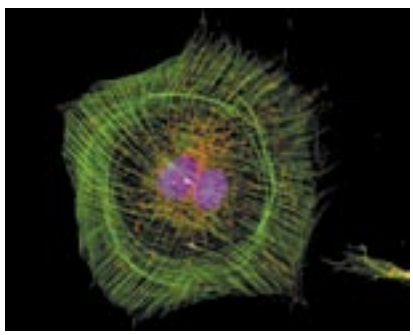


Figure 51. FluoCells prepared slide #6 contains muntjac skin fibroblasts stained with a combination of fluorescent stains. The prominent filamentous actin was labeled with green-fluorescent Alexa Fluor 488 phalloidin. Mitochondria were labeled with an anti–OxPhos Complex V inhibitor protein mouse monoclonal antibody in conjunction with orange-fluorescent Alexa Fluor 555 goat anti–mouse IgG. Nuclei were labeled with the far red–fluorescent TO-PRO-3 iodide nucleic acid stain. This image is a composite of single-wavelength images obtained with filter sets appropriate for FITC, Cy3, and Cy5.

Alexa Fluor Lectin Conjugates

Lectins bind to specific configurations of the various sugar molecules found in cellular glycoproteins. Consequently, lectins are versatile primary detection reagents in histochemical applications (Figures 52 and 53). They are routinely used to identify specific cell and tissue types and to characterize disease and injury states. Our Alexa Fluor lectin conjugates exhibit greater fluorescence intensity and photostability than any other similar dye conjugates (Table 37).

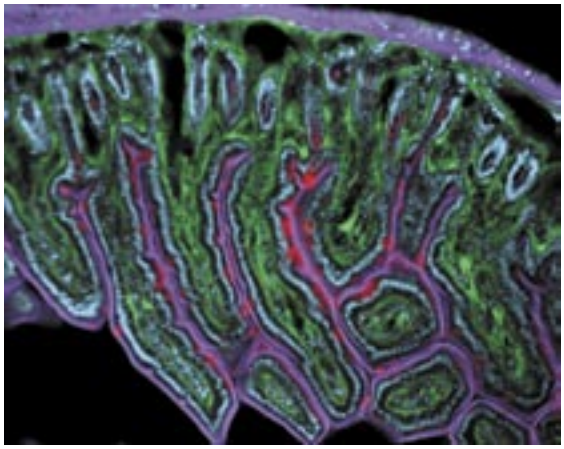


Figure 52. Mouse intestine cryosection showing basement membranes labeled with anti-fibronectin and Alexa Fluor 488 goat anti–chicken IgG antibody (green). Goblet cells and crypt cells were labeled with Alexa Fluor 594 wheat germ agglutinin (red). The microvillar brush border and smooth muscle layer were visualized with Alexa Fluor 680 phalloidin (pseudocolored purple). The section was counterstained with DAPI.

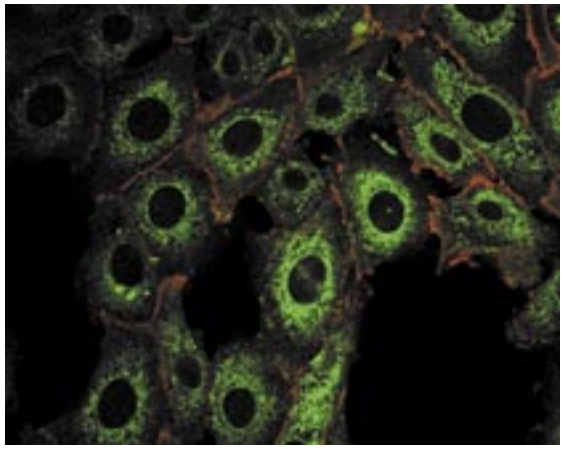


Figure 53. Paraformaldehyde-fixed and Triton X-100–permeabilized MDBK (bovine kidney) cells were labeled with an anti–catenin B rabbit serum and visualized with red-orange–fluorescent Alexa Fluor 546 goat anti–rabbit IgG antibody. The endoplasmic reticulum was stained with the green-fluorescent Alexa Fluor 488 conjugate of concanavalin A. Image contributed by Michal Rychlowski, University of Gdansk.

Table 37. Alexa Fluor lectin conjugates.

Lectin	Alexa Fluor 350	Alexa Fluor 488	Alexa Fluor 568	Alexa Fluor 594	Alexa Fluor 633	Alexa Fluor 647	Alexa Fluor 660	Application
Wheat germ agglutinin (WGA)	W11263	W11261		W11262	W21404			Golgi stain;[1] bacterial gram stain;[2] cell surface glycoprotein expression[3]
Concanavalin A (Con A)	C11254	C11252, C21401*		C11253	C21402	C21421	C21403	Endoplasmic reticulum stain;[1] sugar transport studies;[4] glucose biosensors[5]
Isolectin IB_4 (from an African legume, *Griffonia simplicifolia)*		I21411		I21413		I32450		Microglial cell marker;[6] endothelial cell marker[7]
GS-II (from an African legume, *Griffonia simplicifolia)*		L21415		L21416		L32451		Golgi stain;[8] marker for certain carcinomas[9,10]
PHA-L (from red kidney bean, *Phaseolus vulgaris)*		L11270	L32455	L32456		L32457		Anterograde tracing;[11] marker for certain carcinomas[12,13]
SBA (from soybean, *Glycine max)*		L11272	L32461	L32462		L32463		Carbohydrate expression on cell surfaces[14] and internal membranes[15]
PNA (from peanut, *Arachis hypogaea)*		L21409	L32458	L32459		L32460		Acrosomal marker;[16] marker for certain melanomas[17]

* Succinylated. **1.** J Biol Chem 274, 32975 (1999); **2.** Appl Environ Microbiol 56, 2245 (1990); **3.** Clin Cancer Res 3, 455 (1997); **4.** J Biol Chem 275, 13580 (2000); **5.** Anal Chem 71, 3126 (1999); **6.** Am J Pathol 152, 1307 (1997); **7.** Histochem J 19, 225 (1987); **8.** J Struct Biol 128, 131 (1999); **9.** J Histochem Cytochem 46, 793 (1998); **10.** Histochem J 27, 138 (1995); **11.** Brain Res 854, 122 (2000); **12.** Pathol Int 46, 639 (1996); **13.** Cancer Lett 107, 285 (1996); **14.** Histochem J 29, 583 (1997); **15.** Histochemistry 93, 319 (1990); **16.** Mol Reprod Devel 55, 289 (2000); **17.** Human Pathol 30, 556 (1999).

Alexa Fluor Bioconjugates for Special Applications

Molecular Probes offers a wide selection of quality bioconjugates that combine our proprietary and conventional dyes with antibodies and other molecules. If we do not already have the conjugate you are looking for, you can easily label your own antibody or protein using one of our reactive Alexa Fluor dyes (pages 2–5). We can also prepare a custom conjugate of your antibody, protein, or compound to our fluorescent dyes, biotin, enzymes, or other molecules. See **Custom Synthesis and Bulk Sales** for details.

Alexa Fluor 647 ATP

The **Alexa Fluor 647 conjugate of adenosine 5′-triphosphate** (A22362) links this long-wavelength fluorophore to the ribose via a urethane bridge. This conjugate may be useful in the study of nucleotide-binding proteins.

Alexa Fluor Conjugates of Anti-Hemagglutinin

Our **Alexa Fluor 488** and **Alexa Fluor 594 anti-hemagglutinin (anti–HA) mouse monoclonal antibodies** (A21287, A21288) recognize the influenza hemagglutinin epitope YPYDVPDYA, which has been used extensively as a general epitope tag in expression vectors. As direct conjugates, these antibodies provide a convenient single-step immunolocalization methodology that will produce fluorescent images that have less background than those obtained using the indirect staining methods commonly used.

Alexa Fluor Anti–Glutathione *S*-Transferase Antibody

Another common epitope tag is glutathione *S*-transferase (GST). To facilitate the localization of GST and GST-fusion proteins using immunofluorescence techniques, we prepare the **anti-GST antibody labeled with Alexa Fluor 488 dye** (A11131) . The Alexa Fluor 488 dye, which can be viewed with optical filters appropriate for fluorescein, yields green-fluorescent conjugates that are brighter and much more photostable than fluorescein conjugates.

Fluorescent Fibrinogen

Fluorescently labeled fibrinogen has proven to be a valuable tool for investigating platelet activation and subsequent fibrinogen binding. We offer **human fibrinogen conjugated to** three of our best **Alexa Fluor dyes** (Table 38). These highly fluorescent fibrinogen conjugates may prove useful for investigating platelet activation and subsequent fibrinogen binding using fluorescence microscopy or flow cytometry.

Alexa Fluor Probe for Acrosome Reaction

Trypsin inhibitor from soybean (SBTI) is a 21,000-dalton protein that inhibits the catalytic activity of serine proteases. It has been shown to bind to acrosin, an acrosomal serine protease associated with the binding of spermatozoa and penetration of the zona pellucida. Our **Alexa Fluor 488 dye–labeled SBTI** (T23011) should be useful to researchers monitoring the surface expression of acrosin during the acrosome reaction of fertilization.

Alexa Fluor Methotrexate

Tumor cells often undergo gene amplification that leads to overexpression of dihydrofolate reductase (DHFR). This increased DHFR expression confers enhanced tolerance to the cytotoxic effects of methotrexate. For the study of antimetabolite resistance and spontaneous gene amplification, we offer the green-fluorescent **Alexa Fluor 488 methotrexate** (M23271) to visualize biochemical networks in living cells.

Alexa Fluor cAMP Conjugates

The cyclic AMP (cAMP) analogs used in our **Alexa Fluor cAMP conjugates** (Table 39) are 8-(6-aminohexyl)amino derivatives; such analogs have been shown to exhibit a marked preference for binding to type I cAMP-dependent protein kinases (PKA I).

Table 38. Alexa Fluor fibrinogen conjugates.

Dye	Catalog Number
Alexa Fluor 488	F13191
Alexa Fluor 546	F13192
Alexa Fluor 594	F13193
Alexa Fluor 647	F35200

Table 39. Alexa Fluor cAMP conjugates.

Dye	Catalog Number
Alexa Fluor 488	A35775
Alexa Fluor 555	A35776
Alexa Fluor 647	A35777

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