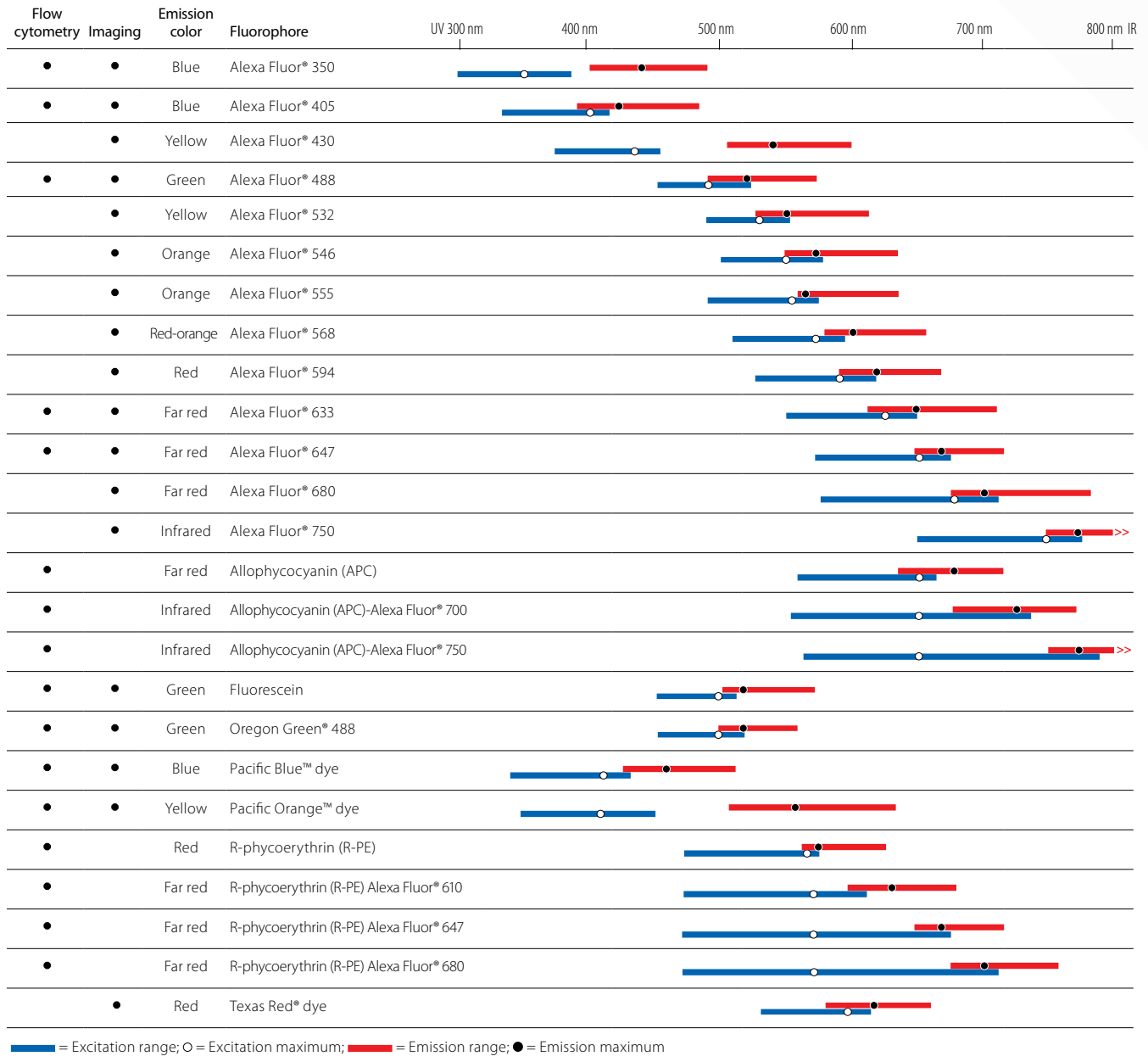


Dye selection guide for antibody labeling kits



Using the Fluorescence Spectra Viewer (www.invitrogen.com/spectraviewer), you can check the spectral compatibility of these and other fluorophores with your instrument and with each other.

Antibody labeling from Z to A...

Invitrogen offers a number of labeling kits for the direct attachment of intensely fluorescent Alexa Fluor® dyes, R-phycoerythrin (R-PE), or even biotin to less than 1 µg to up to 1 mg of IgG antibody. Although the signals from directly labeled antibodies may not be as bright as those observed when using secondary antibodies, their use eliminates the noise commonly observed when secondary antibodies bind nonspecifically to the sample (Figure 1). Also, directly labeled antibodies allow you to use more than one same-species antibody in a single staining experiment. Table 1 will help you choose from Invitrogen's comprehensive selection of innovative, time-tested, and competitively priced antibody and protein labeling kits that feature streamlined protocols for labeling and conjugate purification.

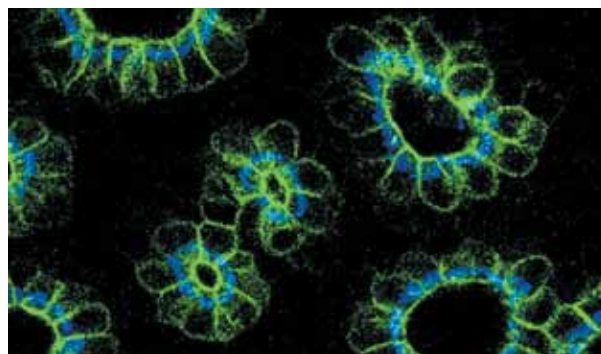


Figure 1. Two-color confocal image of a human epidermal whole mount. A monoclonal antibody to β1 integrin was labeled with Alexa Fluor® 488 dye (green fluorescence, A20181), and α6 integrin was labeled using a primary antibody followed by an Alexa Fluor® 594 secondary (pseudo-colored blue A11005). Image contributed by Uffe Birk Jensen, University of Aarhus, Denmark.

Table 1. IgG antibody labeling kits selection guide.

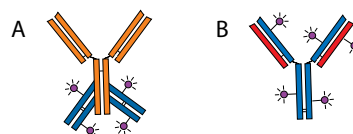
| Amount of IgG | Product | Notes | Attachment | Available fluorophores and labels* | Applications |
|---------------|-------------------------------------|---|-------------|------------------------------------|--------------------------------------|
| <1–20 µg | Zenon® IgG Labeling Kits | <ul style="list-style-type: none"> Antibodies ready to use in 10 min Isotype-specific labeling Compatible with stabilizing proteins | Noncovalent | Organic dyes, phycobiliproteins | FC, ICC |
| 10–20 µg | APEX Antibody Labeling Kits | <ul style="list-style-type: none"> Antibodies ready to use in 2 hr (~15 min hands-on time) Compatible with stabilizing proteins | Covalent | Organic dyes | FC, ICC, IHC |
| 20–100 µg | Microscale Protein Labeling Kits | <ul style="list-style-type: none"> Antibodies ready to use in 2 hr (~30 min hands-on time) Optimized for 10–150 kDa proteins, including IgG antibodies (~150 kDa) Stabilizing proteins must be removed from sample before labeling | Covalent | Organic dyes | FC, ICC, IHC |
| 100 µg | Monoclonal Antibody Labeling Kits | <ul style="list-style-type: none"> Antibodies ready to use in 90 min (~15 min hands-on time) Optimized for both monoclonal and polyclonal IgG antibodies Stabilizing proteins must be removed from sample before labeling | Covalent | Organic dyes | FC, ICC, IHC |
| 0.5–3 mg | SAIMI™ Rapid Antibody Labeling Kits | <ul style="list-style-type: none"> Antibodies ready to use in 75 min (~10 min hands-on time) Includes degree-of-labeling regulator Uses no organic solvents and produces azide-free conjugates | Covalent | Organic dyes | ICC, IHC, <i>in vivo</i> imaging, FC |
| 1 mg | Protein Labeling Kits | <ul style="list-style-type: none"> Antibodies ready to use in 2 hr (~30 min hands-on time) Designed to label monoclonal and polyclonal IgG antibodies Stabilizing proteins must be removed from sample before labeling | Covalent | Organic dyes | FC, ICC, IHC |

FC = flow cytometry; ICC = immunocytochemistry; IHC = immunohistochemistry. Learn more about IgG antibody labeling kits at www.invitrogen.com/ablabeled.

*Organic dyes include fluorophores like Alexa Fluor® dyes as well as fluorescein and tetramethylrhodamine. Phycobiliproteins include R-PE, APC, and their tandems (e.g., Alexa Fluor® 680-R-phycoerythrin (R-PE)).

Covalent vs. noncovalent antibody labeling

Covalent attachment of a label to an antibody generates a stable conjugate—one that lasts several months to years. Labels that are attached noncovalently can dissociate over time—these conjugates last from several hours up to days.



Noncovalent Zenon® antibody labeling (A) vs. covalent microscale, monoclonal, and protein labeling (B).



Labeled antibodies in 10 minutes with Zenon[®] technology

Zenon[®] labeling technology provides a versatile, easy-to-use system for labeling mouse IgG1, IgG2a, IgG2b, and rabbit IgG antibodies with our premier Molecular Probes[®] dyes as well as other fluorophore, biotin, and enzyme labels. This new technology offers several advantages over direct chemical labeling, including:

- **Speed**—the entire labeling procedure takes only 10 min
- **Efficiency**—label nearly 100% of the primary antibody
- **Economy**—label submicrogram amounts of antibody

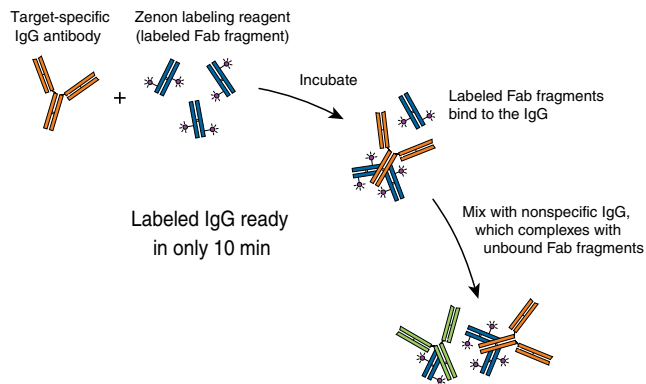


Figure 2. The Zenon[®] labeling scheme. An unlabeled IgG is incubated with the Zenon[®] labeling reagent, which contains a fluorophore-labeled Fab fragment. The labeled Fab fragment binds to the Fc portion of the IgG antibody, and the excess Fab fragment is bound by the addition of a nonspecific IgG. 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 does not need to be coupled to a fluorophore, but could instead be coupled to an enzyme or to biotin.

- **Simplicity**—no pre- or post-labeling purification of the antibody is required
- **Flexibility**—easily use multiple primary antibodies in a single experiment

Our wide selection of Zenon[®] labeling reagents (Tables 2 and 3) can be mixed and matched, providing the freedom to experiment with multiple dye-antibody combinations in flow cytometry and imaging applications.

High affinity and specificity

Zenon[®] labeling technology uses a fluorophore-, biotin-, or enzyme-labeled Fab fragment directed against the Fc portion of an intact IgG antibody to form a labeling complex (Figure 2). To ensure their high affinity and selectivity for the Fc portion of the target antibody, the Zenon[®] labeling reagents have been affinity purified during their preparation. Because the Zenon[®] labeling method is based on immunoselectivity, there is no need to remove exogenous proteins or amine-containing buffers from the antibody sample prior to forming the complex. Antibodies labeled using Zenon[®] technology display fluorescence intensity or enzymatic activity similar to that observed for directly labeled antibody conjugates.

Table 2. Zenon® Tricolor Labeling Kits.*

| | Dyes [†] | Mouse IgG1 | Mouse IgG2a | Mouse IgG2b | Rabbit IgG | Human IgG |
|--------------------------------|---|------------|-------------|-------------|------------|-----------|
| Kit #1 (for imaging) | Alexa Fluor® 488 Alexa Fluor® 555 Alexa Fluor® 647 | Z25060 | Z25160 | Z25260 | Z25360 | Z25460 |
| Kit #2 (for imaging) | Alexa Fluor® 350 Alexa Fluor® 488 Alexa Fluor® 594 | Z25070 | Z25170 | Z25270 | Z25370 | Z25470 |
| Kit #3 (for flow cytometry) | Alexa Fluor® 488 R-phycoerythrin (R-PE) Alexa Fluor® 647-R-PE | Z25080 | Z25180 | Z25280 | Z25380 | |

* Each Zenon® Tricolor Labeling Kit contains materials for 10 labelings with each of the three included labeling reagents. Visit www.invitrogen.com for current pricing. [†] See Table 3 for fluorescence excitation and emission maxima (Ex/Em).

Table 3. Zenon® Labeling Kits.

| | Ex/Em * | Mouse IgG1 | Mouse IgG2a | Mouse IgG2b | Rabbit IgG | Goat IgG | Human IgG |
|--|-----------------------|------------|-------------|-------------|------------|----------|-----------|
| Alexa Fluor® dyes[†] | | | | | | | |
| Alexa Fluor® 350 | 346/442 | Z25000 | Z25100 | Z25200 | Z25300 | | Z25400 |
| Alexa Fluor® 405 | 402/421 | Z25013 | Z25113 | Z25213 | Z25313 | | |
| Alexa Fluor® 430 | 434/539 | Z25001 | | | Z25301 | | |
| Alexa Fluor® 488 | 495/519 | Z25002 | Z25102 | Z25202 | Z25302 | Z25602 | Z25402 |
| Alexa Fluor® 532 | 531/554 | Z25003 | | | Z25303 | | |
| Alexa Fluor® 546 | 556/573 | Z25004 | Z25104 | Z25204 | Z25304 | | |
| Alexa Fluor® 555 | 555/565 | Z25005 | Z25105 | Z25205 | Z25305 | Z25605 | Z25405 |
| Alexa Fluor® 568 | 578/603 | Z25006 | Z25106 | Z25206 | Z25306 | Z25606 | |
| Alexa Fluor® 594 | 590/617 | Z25007 | Z25107 | Z25207 | Z25307 | Z25607 | Z25407 |
| Alexa Fluor® 647 | 650/668 | Z25008 | Z25108 | Z25208 | Z25308 | Z25608 | Z25408 |
| Alexa Fluor® 660 | 663/690 | Z25009 | Z25109 | Z25209 | Z25309 | | |
| Alexa Fluor® 680 | 679/702 | Z25010 | Z25110 | Z25210 | Z25310 | | |
| Alexa Fluor® 700 | 696/719 | Z25011 | | | Z25311 | | |
| Alexa Fluor® 750 | 752/779 | Z25012 | | | Z25312 | | |
| Classic dyes[†] | | | | | | | |
| Pacific Blue™ | 410/455 | Z25041 | Z25156 | | Z25341 | | |
| Pacific Orange™ | 400/551 | Z25256 | Z25257 | | | | |
| Fluorescein | 494/518 | Z25042 | | | Z25342 | | |
| Texas Red®-X | 595/615 | Z25045 | | | Z25345 | | |
| Biotin[†] | | | | | | | |
| Biotin-XX | NA | Z25052 | Z25152 | Z25252 | Z25352 | | Z25452 |
| Phycobiliproteins and tandem dyes[†] | | | | | | | |
| R-phycoerythrin (R-PE) | 496 [§] /578 | Z25055 | Z25155 | Z25255 | Z25355 | | Z25455 |
| Alexa Fluor® 610-R-PE | 496 [§] /630 | Z25020 | | | | | |
| Alexa Fluor® 647-R-PE | 496 [§] /668 | Z25021 | Z25121 | Z25221 | | | |
| Alexa Fluor® 680-R-PE | 496 [§] /702 | Z25022 | | | | | |
| Allophycocyanin (APC) | 650/660 | Z25051 | Z25151 | Z25251 | Z25351 | | Z25451 |
| Alexa Fluor® 700-APC | 650/723 | Z25030 | | | | | |
| Alexa Fluor® 750-APC | 650/775 | Z25031 | | | | | |
| Enzymes[†] | | | | | | | |
| Horseradish peroxidase | NA | Z25054 | Z25154 | Z25254 | Z25354 | | Z25454 |
| Alkaline phosphatase | NA | Z25050 | Z25150 | Z25250 | Z25350 | | |

* Approximate fluorescence excitation and emission maxima, in nm. [†] Each Zenon® Labeling Kit with an Alexa Fluor® dye, classic dye, or biotin contains materials for 50 labelings; one labeling is defined as the amount of Zenon® reagent required to label 1 µg of antibody. [‡] Each Zenon® Labeling Kit with a phycobiliprotein or enzyme contains materials for 25 labelings; kits with tandem dyes contain materials for 10 labelings. One labeling is defined as the amount of Zenon® reagent required to label 1 µg of antibody. [§] Additional excitation peaks are present at 546 and 565 nm. NA = Not applicable. Visit www.invitrogen.com for pricing.



Zenon® imaging applications

Zenon® labeling technology simplifies time-consuming immunocytochemical applications such as those that use multiple same-species-derived primary antibodies in one staining experiment (Figures 3, 4, and 5). The extensive selection of available labels and the versatility of Zenon® technology makes it easy to experiment

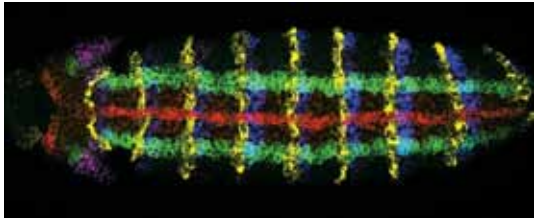


Figure 3. Simultaneous detection of expression of five genes in a whole-mount *Drosophila* embryo by fluorescence *in situ* hybridization (FISH) with five RNA probes. Red: *sog* labeled using aminoallyl UTP and Alexa Fluor® 647 succinimidyl ester (A20006). Green: *ind* labeled with DNP, followed by rabbit anti-dinitrophenyl-KLH antibody (A6430) prelabeled with the Zenon® Alexa Fluor® 555 Rabbit IgG Labeling Kit (Z25305). Blue: *en* labeled with biotin and detected with HRP-streptavidin and Alexa Fluor® 405 tyramide. Yellow: *wg* labeled with digoxigenin and detected with sheep anti-digoxigenin antibody and Alexa Fluor® 594 donkey anti-sheep IgG antibody (A11016). Magenta: *msh* labeled with fluorescein and detected with mouse anti-fluorescein/Oregon Green® antibody (A6421) and Alexa Fluor® 488 goat anti-mouse IgG antibody (A11001). Image contributed by Dave Kosman and Ethan Bier, University of California, San Diego.

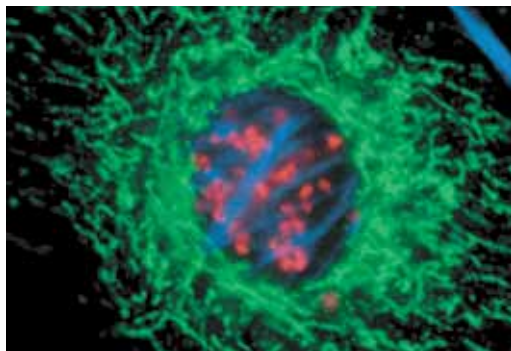


Figure 4. Bovine pulmonary artery endothelial cells labeled with probes for actin, mitochondria, and the phosphorylated form of histone H3. Actin was labeled with blue-fluorescent Alexa Fluor® 350 phalloidin (A22281), and phosphorylated histone H3 was detected using a rabbit anti-phosphohistone H3 antibody prelabeled with the Zenon® Alexa Fluor® 555 Rabbit IgG Labeling Kit (red fluorescence, Z25305). Endogenous biotin associated with the mitochondria was labeled using streptavidin (S888) followed by a rabbit anti-streptavidin antibody prelabeled with the Zenon® Alexa Fluor® 488 Rabbit IgG Labeling Kit (green fluorescence, Z25302).

with different color combinations for optimal multicolor images. More examples of Zenon® technology in action can be seen in our online image gallery at www.invitrogen.com/zenon.

Our Zenon® Labeling Kits are currently available with the following labels:

- Alexa Fluor® dyes—our premier fluorescent dyes
- Classic fluorescent labels—fluorescein and Pacific Blue™, Pacific Orange™, and Texas Red®-X dyes
- Biotin—for use with avidin-based secondary detection reagents
- Allophycocyanin and R-phycoerythrin—popular labels for flow cytometry applications
- Alkaline phosphatase and horseradish peroxidase—for use with signal amplification systems such as TSA, ELF, and DAB

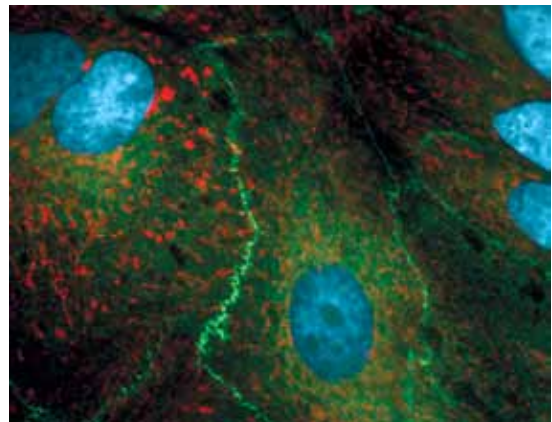


Figure 5. Madin-Darby canine kidney (MDCK) cells showing the distribution of the tight-junction protein ZO-3 and mitochondria. ZO-3 was detected with a rabbit anti-ZO-3 antibody prelabeled with the Zenon® Alexa Fluor® 488 Rabbit IgG Labeling Kit (Z25302). Endogenous biotin in the mitochondria was labeled using streptavidin (S888) followed by a rabbit anti-streptavidin antibody prelabeled with the Zenon® Alexa Fluor® 555 Rabbit IgG Labeling Kit (Z25305). The nuclei were stained with DAPI (D1306, D3571, D21490).

Zenon® labeling for flow cytometry applications

Zenon® labeling technology is quick and convenient, and requires only 1 µg of primary antibody for the entire labeling procedure. The resulting Zenon® staining complex is fully compatible with most flow cytometry applications and offers the following features:

- Suitable for multicolor applications (Figure 6)
- Brightness comparable to direct antibody conjugates (Figure 7)
- Compatible with live or fixed cultured cell samples
- Compatible with whole blood samples—staining can be performed either before or after lysis of red blood cells
- Avoids the multiple steps involved with indirect staining procedures, especially in multicolor applications
- Eliminates the added time and expense of an Fc-blocking step
- Color combinations can be changed easily

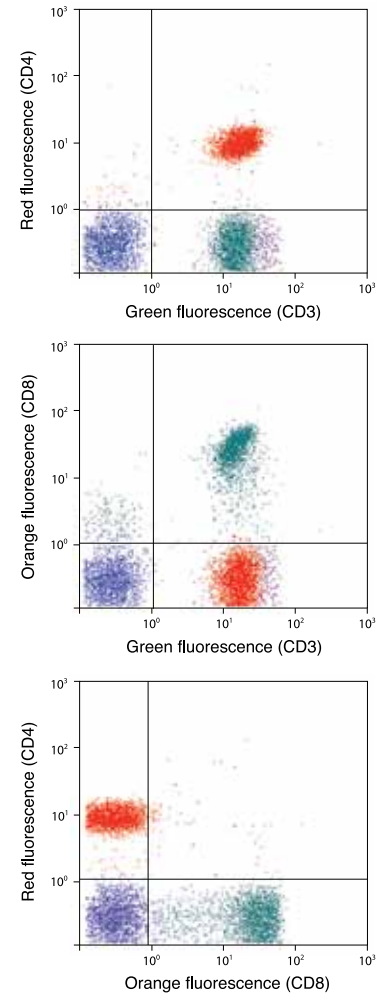


Figure 6. Human peripheral blood mononuclear cells stained with markers for CD3, CD4, and CD8, and detected using a lymphocyte gate. The cell sample was stained with an anti-CD3 mouse IgG1 antibody pre-labeled using the Zenon® Alexa Fluor® 488 Mouse IgG1 Labeling Kit, an anti-CD4 mouse IgG1 antibody pre-labeled using the Zenon® Alexa Fluor® 647-R-Phycoerythrin Mouse IgG1 Labeling Kit, and an anti-CD8 mouse IgG2a antibody pre-labeled using the Zenon® Alexa Fluor® 647-R-Phycoerythrin Mouse IgG2a Labeling Kit. Plots of CD3 vs. CD4 staining (top), CD3 vs. CD8 staining (middle), and CD8 vs. CD4 staining (bottom) all demonstrate good signal separation and the expected differentiation of CD4⁺ and CD8⁺ cells. The samples were analyzed on a Coulter Elite™ flow cytometer using excitation at 488 nm and bandpass emission filters appropriate for the detection of Alexa Fluor® 488 dye, R-phycoerythrin, and the Alexa Fluor® 647-R-phycoerythrin tandem dye.

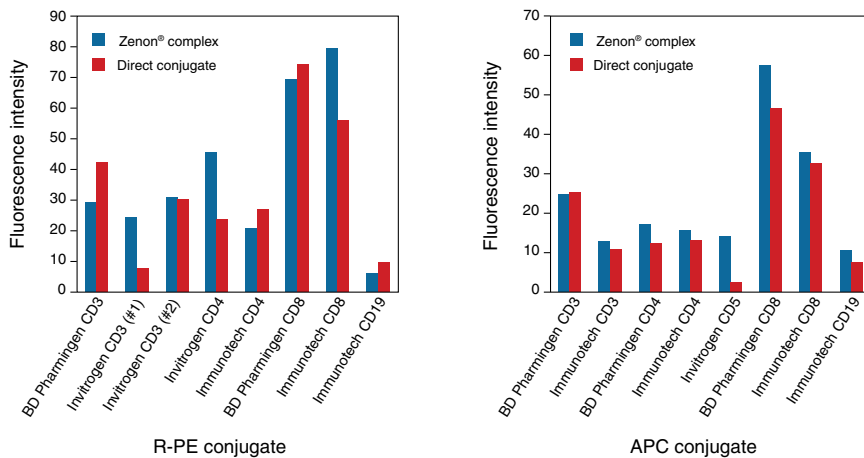


Figure 7. Brightness comparison of phycoerythrin (R-PE) and allophycocyanin (APC) direct antibody conjugates with the comparable Zenon® staining complexes. Human peripheral blood lymphocytes were incubated with a directly labeled antibody conjugate against the indicated CD marker according to the manufacturer's recommendations. In a parallel experiment, lymphocytes were incubated with 1 µg of a Zenon® labeling complex formed using the unconjugated version of the same antibody, prepared according to the Zenon® labeling kit protocol. In most cases, the Zenon® staining complex produced brightness comparable to or more intense than that obtained from the direct conjugate. The brightness of the Zenon® staining complex can be further enhanced by increasing the ratio of Zenon® labeling reagent to the primary antibody used in the preparation of the complex.



Covalent antibody labeling kits with fluorescent dyes or biotin

Zenon® technology is great to quickly label small amounts (<1 µg and up to 20 µg) of IgG antibody. However, the complex rapidly formed between the IgG antibody and the dye-labeled Zenon® Fab fragment directed against the Fc portion of the IgG is noncovalent and can slowly dissociate over time. As a result, Zenon® antibody labeling is not recommended for applications requiring long or overnight incubations. Furthermore, the Zenon® kits are not designed to label larger amounts of IgG (>20 µg). The antibody labeling kits discussed below (Tables 4 and 6) directly and covalently attach an amine-reactive fluorophore or biotin label to the IgG antibody. The strong bond between the label and the antibody is extremely stable and is formed by the same chemistry employed to prepare our own primary and secondary antibody conjugates.

APEX Antibody Labeling Kits

Many IgG antibodies are available only in small quantities and packaged with stabilizing proteins, such as bovine serum albumin (BSA), or other contaminants. These contaminants can interfere with the amine-reactive labeling reagents used to covalently attach a fluorophore to an antibody, and removing these contaminants can cause significant antibody loss. The new APEX Antibody Labeling Kits provide a convenient means to directly attach a fluorophore to very small amounts of IgG antibody (10–20 µg), while allowing you to easily remove contaminants without losing antibody.

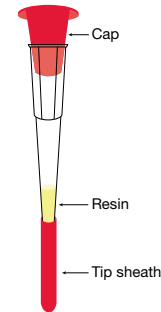


Figure 8. APEX Antibody Labeling Kits allow convenient elution of contaminants through the filter tip.

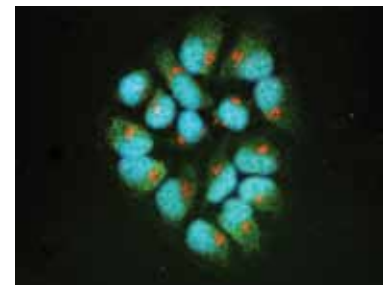


Figure 9. Mitochondrial and Golgi complex labeling in HeLa cells. Fixed and permeabilized HeLa cells were treated with multiple mouse primary antibodies directly conjugated with APEX Alexa Fluor® Antibody Labeling Kits. The Golgi complex was detected with an anti-golgin-97 mouse monoclonal antibody labeled using the APEX Alexa Fluor® 555 Antibody Labeling Kit (A10470, orange fluorescence). Mitochondria were detected with an anti-OxPhos Complex V inhibitor protein mouse monoclonal antibody labeled using the APEX Alexa Fluor® 488 Antibody Labeling Kit (A10468, green fluorescence). Nuclei were stained with blue-fluorescent DAPI (D1306, D3571, D21490).

Table 4. Invitrogen's IgG antibody covalent labeling kits.

| IgG antibody labeling kit | Number of reactions | Amount labeled/ reaction | Sample requirements | Purification method |
|------------------------------------|---------------------|--------------------------|--|---|
| APEX Antibody Labeling Kit | 5 | 10–20 µg of IgG | Reaction takes place in <10 µL; completely compatible with BSA or other stabilizing proteins and azide | Size exclusion chromatography |
| Microscale Protein Labeling Kit | 3 | 20–100 µg of IgG* | No BSA or other stabilizing proteins; no azide | Size exclusion chromatography, spin filter |
| Monoclonal Antibody Labeling Kit | 5 | 100 µg of IgG | No BSA or other stabilizing proteins; no azide | Size exclusion chromatography, spin column |
| SAIVI™ Rapid Antibody Labeling Kit | 3 | 0.5–3.0 mg of IgG | No BSA or other stabilizing proteins; no azide | Size exclusion chromatography, gravity column |
| Protein Labeling Kit | 3 | 1 mg of IgG | No BSA or other stabilizing proteins; no azide | Size exclusion chromatography, gravity column |

* Other proteins between 10 and 150 kDa can also be labeled with this kit.

Lose contaminants without sacrificing antibody

APEX Antibody Labeling Kits utilize a solid-phase labeling technique that captures the IgG antibody on the resin inside the APEX antibody labeling tip (Figure 8). This enables covalent labeling of antibodies that are supplied in solutions containing stabilizing proteins (i.e., BSA) or other contaminants, which can interfere with the amine-reactive labeling reagents that are used to attach the fluorophore to the antibody. With the APEX Antibody Labeling Kit technology, contaminants are simply eluted through the tip. After applying the amine-reactive label, the fluorescent IgG conjugate is ready for use in an imaging (Figures 9 and 10) or flow cytometry application in as little as 2 hours with very little hands-on time.

Eliminate background fluorescence, gain flexibility

Although secondary antibodies offer a brighter fluorescent signal, directly labeled antibodies eliminate background fluorescence commonly observed when secondary antibodies bind nonspecifically to the sample. In addition, directly labeled antibodies allow more than one same-species antibody in a single staining experiment.

APEX Antibody Labeling Kits include all the reagents necessary to perform five separate labeling reactions of 10–20 µg of IgG antibody with superior Molecular Probes® fluorophores, some of which can be used as alternatives to biotin (Table 5). Unlike biotin, which is an endogenous ligand in mitochondria, dye-based haptens permit background-free staining of cells and tissues. Other available Molecular Probes® antibody and protein labeling kits are optimized for covalent labeling of larger amounts of IgG antibodies or proteins.

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 between 12 and 150 kDa, and contain everything needed to perform labeling reactions and to separate the resulting conjugates from excess dye. Convenient spin columns are used to purify the labeled protein (Figure 11) with yields of 60–90%, depending primarily on the molecular weight of the starting material. Labeling and purification can be completed in as little as 30 minutes.

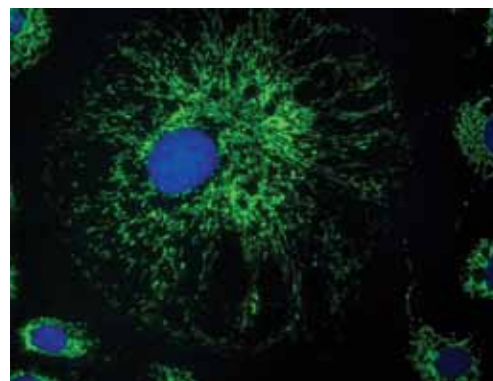


Figure 10. Mitochondrial labeling in bovine pulmonary artery endothelial (BPAE) cells. Fixed and permeabilized BPAE cells were treated with an anti-OxPhos Complex V inhibitor protein mouse IgG1 monoclonal antibody labeled using the APEX Alexa Fluor® 488 Antibody Labeling Kit (Cat. No. A10468, green fluorescence) to detect mitochondria. Nuclei were stained with blue-fluorescent DAPI (Cat. No. D1306, D3571, D21490).

Table 5. Spectral characteristics and applications of the fluorescent labels available in the APEX Antibody Labeling Kits.

| Label | Abs* | Em* | Application |
|-------------------|------|-----|--|
| Alexa Fluor® 488 | 495 | 518 | Fluorescent label for use in imaging or flow cytometry; hapten for signal amplification with anti-Alexa Fluor® 488 antibodies |
| Alexa Fluor® 555 | 555 | 565 | Fluorescent label for use in imaging |
| Alexa Fluor® 594 | 590 | 617 | Fluorescent label for use in imaging |
| Alexa Fluor® 647 | 650 | 665 | Fluorescent label for use in imaging or flow cytometry |
| Oregon Green® 488 | 496 | 524 | Fluorescent label for use in imaging or flow cytometry; hapten for signal amplification with anti-fluorescein/Oregon Green® antibodies |
| Pacific Blue™ | 416 | 451 | Fluorescent label for use in imaging or flow cytometry |

* Absorption (Abs) or fluorescence emission (Em) maxima, in nm.

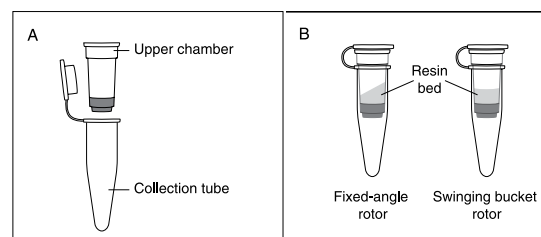


Figure 11. Molecular Probes® Microscale Antibody Labeling Kit Spin filter. (A) An empty filter showing the separate parts. (B) Appearance of the resin bed after centrifugation in a fixed-angle rotor or swinging bucket rotor.



Monoclonal Antibody Labeling Kits

Molecular Probes® Monoclonal Antibody Labeling Kits 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 a vial of amine-reactive dye. Purification is accomplished on a size exclusion spin column optimized for ≥40 kDa proteins (Figure 12).

SAIVI™ Rapid Antibody Labeling Kits

Molecular Probes® SAIVI™ Rapid Antibody Labeling Kits provide a quick and simple way to label antibodies with an Alexa Fluor® 680 or Alexa Fluor® 750 near-infrared dye. The optimized protocol provides rigorous control over the degree of labeling (DOL; ~2) over a 6-fold antibody concentration range and produces labeled antibodies that are optimized for *in vivo* imaging (Figure 13). SAIVI™ Rapid Antibody Labeling Kits include a novel column that has an easy-to-use premeasured slurry for a 10-minute prep time and short wash step. No organic solvents are used in the labeling protocol, and purification of the dye-labeled conjugate is achieved with a rapid, simple protocol, which produces excellent reproducibility. SAIVI™ Rapid Antibody Labeling Kits are optimized to label 0.5 to 3.0 mg of antibody in less than 90 minutes (with only 10 minutes of hands-on time), and the resulting labeled antibody is in an azide-free buffer suitable for live-cell imaging or direct injection into animals.

Protein Labeling Kits

Our easy-to-use Protein Labeling Kits 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 vials containing a premeasured quantity of amine-reactive dye and a magnetic stir bar. Purification is accomplished on a gravity-feed size exclusion column supplied with the kit (Figure 14). Although optimized for IgG antibodies, the kits can be used to make conjugates of other proteins larger than ~40 kDa.

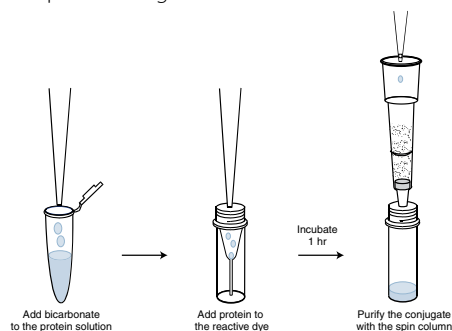


Figure 12. Molecular Probes® Monoclonal Antibody Labeling Kits are the simplest way to label 100 µg of IgG antibodies.

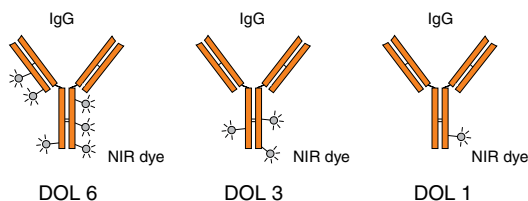


Figure 13. SAIVI™ labeling kits provide DOL control. A simple protocol for varying the degree of labeling (DOL) allows for optimization of brightness and specificity in *in vivo* applications.

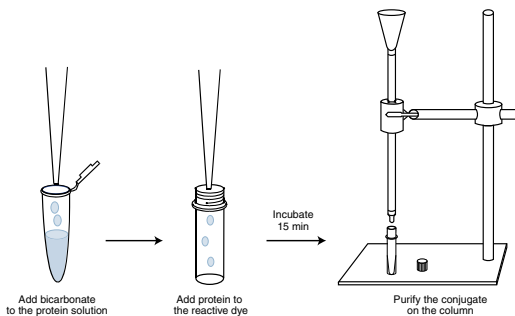


Figure 14. Molecular Probes® Protein Labeling Kits are the simplest way to label 1 mg of IgG antibodies.

Table 6. Antibody covalent labeling kits.

| Fluorophore | Ex/Em* | 10–20 µg IgG APEX Antibody Labeling Kits | 20–100 µg Microscale Protein Labeling Kits | 100 µg Monoclonal Anti-body Labeling Kits | 0.5–3 mg SAIVI™ Rapid Anti-body Labeling Kits | 1 mg Protein Labeling Kits |
|-----------------------|-----------------------|--|--|---|---|----------------------------|
| Alexa Fluor® 350 | 346/442 | | | A20180 | | A10170 |
| Alexa Fluor® 405 | 402/421 | | | | | |
| Alexa Fluor® 430 | 433/539 | | | | | |
| Alexa Fluor® 488 | 495/518 | A10468 | A30006 | A20181 | | A10235 |
| Alexa Fluor® 532 | 530/554 | | | A20182 | | A10236 |
| Alexa Fluor® 546 | 556/575 | | | A20183 | | A10237 |
| Alexa Fluor® 555 | 555/565 | A10470 | A30007 | A20187 | | A20174 |
| Alexa Fluor® 568 | 578/603 | | | A20184 | | A10238 |
| Alexa Fluor® 594 | 590/617 | A10474 | A30008 | A20185 | | A10239 |
| Alexa Fluor® 633 | 621/639 | | | | | A20170 |
| Alexa Fluor® 647 | 650/665 | A10475 | A30009 | A20186 | S30044 | A20173 |
| Alexa Fluor® 660 | 663/690 | | | | | A20171 |
| Alexa Fluor® 680 | 679/702 | | | | S30045 | A20172 |
| Alexa Fluor® 700 | 696/719 | | | | | |
| Alexa Fluor® 750 | 749/775 | | | | S30046 | |
| APC | 650/660 | | | | | |
| APC–Alexa Fluor® 700 | 650/723 | | | | | |
| APC–Alexa Fluor® 750 | 650/775 | | | | | |
| Biotin | NA | | B30010 | | | D20655 |
| Fluorescein | 494/518 | | | | | F10240 |
| Oregon Green® 488 | 496/524 | A10476 | | | | O10241 |
| Pacific Blue™ | 416/451 | A10478 | | P30013 | | P30012 |
| Pacific Orange™ | 400/551 | | | P30014 | | P30016 |
| R-PE | 496 [†] /578 | | | | | |
| R-PE–Alexa Fluor® 610 | 496 [†] /630 | | | | | |
| R-PE–Alexa Fluor® 647 | 496 [†] /668 | | | | | |
| R-PE–Alexa Fluor® 680 | 496 [†] /702 | | | | | |
| Texas Red®-X | 595/615 | | | | | T10244 |

* Approximate fluorescence excitation and emission maxima, in nm. † Additional excitation peaks are present at 546 and 565 nm.

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Image-iT® FX Signal Enhancer dramatically improves the signal-to-noise ratio of immunolabeled cells and tissues, allowing you to clearly visualize targets that would normally be indistinguishable. Background staining typically seen with fluorescent dyes is largely eliminated when Image-iT® FX Signal Enhancer is applied to fixed and permeabilized cells (Figure 15).

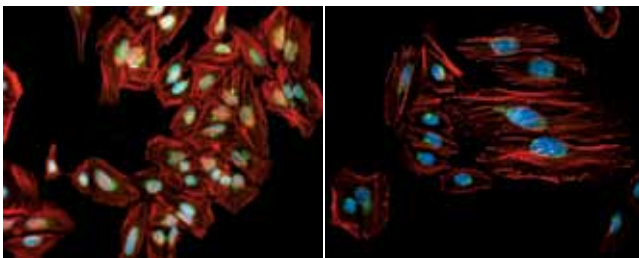


Figure 15. Increased label specificity and resolution provided by Image-iT® FX Signal Enhancer. Golgi in fixed and permeabilized HeLa cells labeled with anti-golgin-97 (A21270) and visualized with green-fluorescent Alexa Fluor® 488 goat anti-mouse IgG (A11001). Actin was stained with red-fluorescent Alexa Fluor® 594 phalloidin (A12381); nuclei were stained with blue-fluorescent DAPI (D3571). Cells in the right panel were treated with Image-iT® FX Signal Enhancer (I36933), which eliminates nonspecific dye binding.

Protect your samples from photobleaching with ProLong® and SlowFade® Gold Antifade Reagents

ProLong® Gold Antifade Reagent outperforms most other commercially available antifade reagents, significantly reducing fluorophore photobleaching while causing little or no quenching of the fluorescence signal (Figures 16 and 17). This reagent offers excellent compatibility with a multitude of dyes and dye complexes, making it an especially valuable tool for multicolor applications.

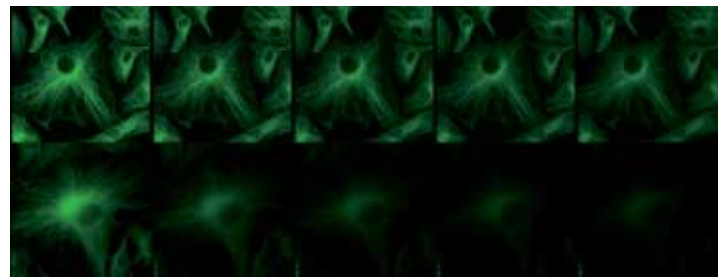


Figure 16. Enhanced resistance to photobleaching afforded by ProLong® Gold Antifade Reagent. Fixed bovine pulmonary artery endothelial cells were labeled with anti- α -tubulin (A11126) and visualized with fluorescein goat anti-mouse IgG (F2761). The samples were mounted in ProLong® Gold Antifade Reagent (P36930; top) or phosphate-buffered saline (bottom). Images were acquired at 5 sec intervals for a total of 20 sec using a 40x/1.3 NA oil immersion objective with continuous illumination from a standard 100 W Hg-arc lamp.

ProLong® Gold Antifade Reagent is premixed and ready to use—just add a drop to your preparation and mount. ProLong® Gold reagent cures within 24 hours, and the sample can be saved for months after mounting. ProLong® Gold Antifade Reagent is also available with DAPI nucleic acid stain included in the formulation.

- Premixed and ready to use
- Cures within 24 hr
- Samples can be saved for months after mounting

SlowFade® Gold Antifade Reagent offers increased resistance to photobleaching for a wide range of fluorescent dyes (Figure 18). Unlike ProLong® Gold reagent, *SlowFade*® Gold reagent does not cure over time, so samples can be viewed immediately—simply tack the corners of the slide with hot wax or nail polish, then image.

- Premixed and ready to use
- Ideal for short-term use

Molecular Probes® signal enhancer and antifade reagents.

| Product | Quantity | Cat. No. |
|--|----------|----------|
| Image-iT® FX Signal Enhancer | 10 mL | I36933 |
| ProLong® Gold Antifade Reagent | 10 mL | P36930 |
| ProLong® Gold Antifade Reagent, special packaging | 5 x 2 mL | P36934 |
| ProLong® Gold Antifade Reagent with DAPI | 10 mL | P36931 |
| ProLong® Gold Antifade Reagent with DAPI, special packaging | 5 x 2 mL | P36935 |
| <i>SlowFade</i> ® Gold Antifade Reagent | 10 mL | S36936 |
| <i>SlowFade</i> ® Gold Antifade Reagent, special packaging | 5 x 2 mL | S36937 |
| <i>SlowFade</i> ® Gold Antifade Reagent with DAPI | 10 mL | S36938 |
| <i>SlowFade</i> ® Gold Antifade Reagent with DAPI, special packaging | 5 x 2 mL | S36939 |

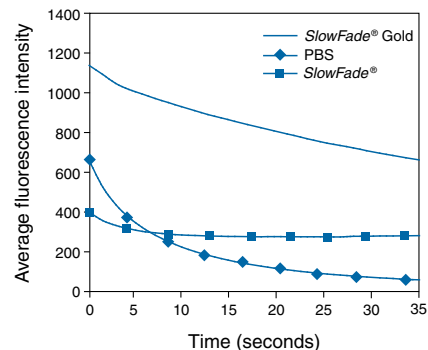


Figure 17. *SlowFade*® Gold Antifade Reagent provides enhanced resistance to photobleaching. Fluorescein-labeled microspheres were mounted with the specified antifade reagents and imaged. Data represent the average fluorescence intensity from 20 microspheres.

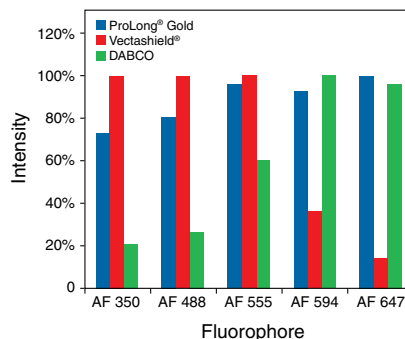
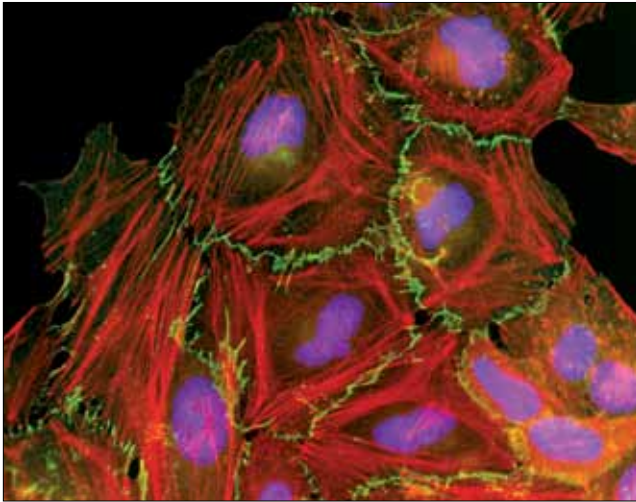
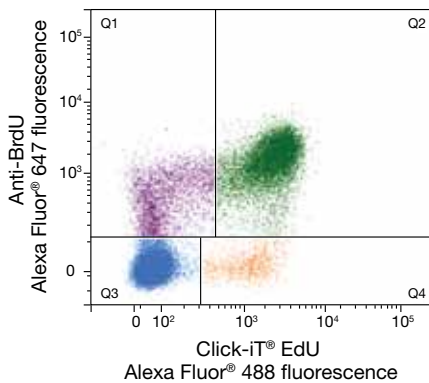


Figure 18. ProLong® Gold Antifade Reagent outperforms competitors’ antifades across the visible spectrum. While competitors’ reagents preserve signal strength at only specific wavelengths, ProLong® Gold reagent offers stability across the entire spectrum. AF = Alexa Fluor®.



α -catenin in HeLa human cervical cancer cells labeled using mouse anti- α -catenin and visualized with Alexa Fluor® 488 goat anti-mouse IgG (green). Filamentous actin was visualized using red-fluorescent Alexa Fluor® 635 phalloidin. Nuclear DNA was stained with blue-fluorescent DAPI.



Dual-pulse labeling of Jurkat cells with EdU and BrdU as analyzed by flow cytometry. EdU was detected with Alexa Fluor® 488 azide using the Click-iT® EdU Flow Cytometry Kit (Cat. No. C35002). BrdU was then detected with anti-BrdU, Alexa Fluor® 647 conjugate (Cat. No. A21305). SYTOX® Blue nucleic acid stain (Cat. No. S11348) with RNase was used to detect DNA content. Four populations of cells are distinguished in the EdU vs. BrdU plot: cells positive for both (Q2), cells negative for both (Q3), EdU-positive only (Q4), and BrdU-positive only (Q1).

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NOTES



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