

## Novel solid-phase antibody labeling

TAKE ADVANTAGE OF THE SPEED, EFFICIENCY, AND CONVENIENCE OF APEX ANTIBODY LABELING KITS.

Many IgG antibodies are available only in small quantities and are 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 of directly labeling a very small amount of IgG antibody (10–20 µg) with a fluorophore, while allowing you to easily remove contaminants without losing antibody.

### Lose contaminants without sacrificing antibody

The APEX Antibody Labeling Kits use a solid-phase labeling technique that captures the IgG antibody on the resin inside the APEX antibody labeling tip (Figure 1). Any contaminants, including stabilizing proteins or amine-containing buffers, are simply eluted through the tip. After applying the amine-reactive label, a fluorescent IgG conjugate is ready for use in an imaging (Figures 2 and 3) or flow cytometry application in as little as 2.5 hours with very little hands-on time.

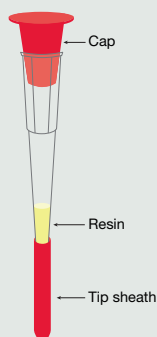


Figure 1—Diagram of an APEX antibody labeling tip.

### Eliminate background fluorescence, gain flexibility

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

### Start labeling today

The APEX antibody labeling kits include all of 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 1). 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 noncovalent labeling of smaller or larger amounts of IgG antibodies or proteins >30 kDa (Table 2). Learn more at [www.invitrogen.com/bp58](http://www.invitrogen.com/bp58). ■

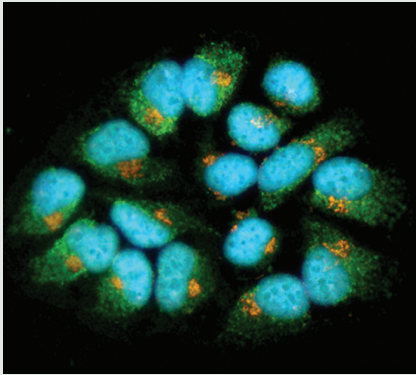
Table 1—Spectral characteristics and applications of the fluorescent labels available in the APEX Antibody Labeling Kits.

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

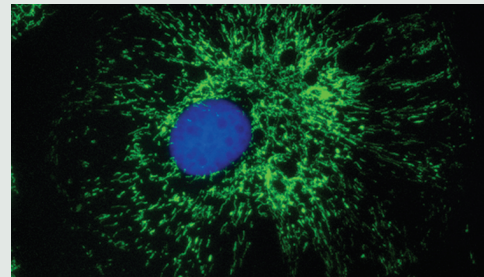
\* Absorption (Abs) or fluorescence emission (Em) maxima, in nm.

**Table 2—Molecular Probes® antibody and protein labeling kits.**

Amount of IgG	Product	Key features
<1–20 µg	Zenon® IgG Labeling Kits	<ul style="list-style-type: none"> <li>Labeled IgG antibodies ready to use in 10 min</li> <li>Isotype-specific labeling</li> <li>Fast, noncovalent attachment of label</li> <li>Labeling compatible with stabilizing proteins such as BSA</li> </ul>
10–20 µg	APEX Antibody Labeling Kits	<ul style="list-style-type: none"> <li>Labeled IgG antibodies ready to use in 2.5 hr (~15 min hands-on time)</li> <li>Covalent attachment of label</li> <li>Labeling compatible with stabilizing proteins such as BSA</li> </ul>
20–100 µg	Microscale Protein Labeling Kits	<ul style="list-style-type: none"> <li>Labeled antibodies or other proteins ready to use in 2 hr (~30 min hands-on time)</li> <li>Covalent attachment of label</li> <li>Optimized for proteins between 10 and 150 kDa, including IgG antibodies (~150 kDa)</li> <li>Stabilizing proteins must be removed from sample before labeling</li> </ul>
100 µg	Monoclonal Antibody Labeling Kits	<ul style="list-style-type: none"> <li>Labeled IgG antibodies ready to use in 90 min (~15 min hands-on time)</li> <li>Covalent attachment of label</li> <li>Optimized for IgG antibodies (~150 kDa)</li> <li>Stabilizing proteins must be removed from sample before labeling</li> <li>Designed to label polyclonal and monoclonal IgG antibodies</li> </ul>
1 mg	Protein Labeling Kits	<ul style="list-style-type: none"> <li>Labeled antibodies ready to use in 2 hr (~30 min hands-on time)</li> <li>Covalent attachment of label</li> <li>Optimized for IgG antibodies (~150 kDa)</li> <li>Stabilizing proteins must be removed from sample before labeling</li> </ul>
0.3–5 mg	SAIMI™ Antibody Labeling Kits	<ul style="list-style-type: none"> <li>Labeled IgG antibodies ready to use in 90 min (~10 min hands-on time)</li> <li>Covalent attachment of label</li> <li>Optimal degree of labeling for <i>in vivo</i> imaging applications</li> <li>Labeled antibodies ready for use in applications that require azide-free reagents, such as live-cell imaging or direct injection into animals</li> </ul>



**Figure 2—Mitochondrial and Golgi complex labeling in HeLa cells.** Fixed and permeabilized HeLa cells were treated with multiple mouse primary antibodies directly conjugated using APEX Alexa Fluor® Antibody Labeling Kits. The Golgi apparatus was detected with an anti-golgin-97 mouse monoclonal antibody labeled using the APEX Alexa Fluor® 555 Antibody Labeling Kit (Cat. no. A10470, orange fluorescence). Mitochondria were detected 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). Nuclei were stained with blue-fluorescent DAPI (Cat. no. D1306, D3571, D21490).



**Figure 3—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).

Product	Quantity	Cat. no.
APEX Alexa Fluor® 488 Antibody Labeling Kit	1 kit	A10468
APEX Alexa Fluor® 555 Antibody Labeling Kit	1 kit	A10470
APEX Alexa Fluor® 594 Antibody Labeling Kit	1 kit	A10474
APEX Alexa Fluor® 647 Antibody Labeling Kit	1 kit	A10475
APEX Oregon Green® 488 Antibody Labeling Kit	1 kit	A01476
APEX Pacific Blue™ Antibody Labeling Kit	1 kit	A10478