APEX Antibody Labeling Kits

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
Reactive fluorescent label (Component A)	5 vials	Not applicable	• 2–6°C • Desiccate • Protect from light • **Do not freeze**	When stored as directed this kit is stable for 6 months.
APEX antibody labeling tips (Component B)	5 each			
wash buffer (Component C)	1.8 mL	0.1 M phosphate buffered saline (PBS), pH 7.5, 2 mM azide		
dimethylsulfoxide (DMSO, Component D)	100 µL	Not applicable		
labeling buffer (Component E)		50 mM borate buffer, pH 8.3		
neutralization buffer (Component F)		1 M Tris, pH 9.0		
elution buffer (Component G)	1 mL	0.2 M acetic acid, pH 3.3		

*These storage conditions are appropriate when storing the entire kit upon receipt. For optimal storage conditions for each component, see individual component labels.

Number of assays: Sufficient material is supplied for 5 labelings of 10–20 µg of IgG antibody based upon the protocol below.

Approximate fluorescence excitation/emission maxima: See Table 2.

Introduction

Invitrogen's APEX Antibody Labeling Kits provide a convenient method to covalently attach a fluorophore to small amounts of IgG antibody (approximately, 10–20 µg). A primary antibody directly labeled with a fluorophore often produces lower background fluorescence and less nonspecific binding. Furthermore, multiple primary antibodies of the same isotype or derived from the same species can easily be used in the same experiment if they are directly labeled with compatible fluorophores. Many IgG antibodies are often available only in small quantities and packaged with stabilizing proteins, such as BSA, or other contaminants which can interfere with the amine-reactive labeling reagents commonly used to covalently attach the fluorophore to the antibody. Removal of these contaminants often result in significant loss of the IgG antibody.

The 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 1). Any contaminants, including stabilizing proteins or amine-containing buffers are eluted through the tip. After applying the amine-reactive, fluorescent label to the IgG antibody on the resin, a fluorescent IgG conjugate is formed and is eluted from the resin using elution buffer. The fluorescent IgG conjugate is ready for use in an imaging or flow cytometry assay in as little as 2.5 hours with minimal hands on time. The typical yield of labeled antibody using this

method ranges from 40–80%.

The APEX Antibody Labeling Kits includes all reagents required to perform 5 separate labeling reactions of 10–20 µg of IgG antibody with one of Molecular Probes' superior fluorophores, some of which can also be utilized as an alternative to biotin (Table 2). Unlike biotin, which is an endogenous ligand in mitochondria, dye-based haptens permit background-free staining of cells and tissues.

Before you begin

Materials Required but Not Provided

- 100–200 µL pipette
- 10–20 µg IgG antibody in ≤10 µL in neutral pH buffer such as phosphate buffered saline (PBS), Tris-buffered saline (TBS), Tris-HCl, HEPES, borate, or equivalent (the sample can contain serum or other stabilizing proteins)
- Microcentrifuge tubes (2 per reaction)

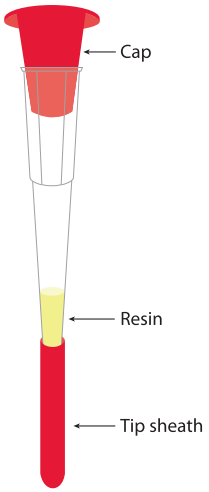

Figure 1. APEX antibody labeling tip.

Table 2. Spectral characteristics and applications of the labels available in the APEX Antibody Labeling Kits.

Fluorophore label	Cat. no.	Excitation (nm)	Emission (nm)	Application
Alexa Fluor® 488	A10468	495	518	Fluorescent label for use in imaging or flow cytometry; Hapten for signal amplification with anti-Alexa Fluor® 488 antibodies.
Alexa Fluor® 555	A10470	555	565	Fluorescent label for use in imaging.
Alexa Fluor® 594	A10474	590	617	
Alexa Fluor® 647	A10475	650	665	Fluorescent label for use in imaging or flow cytometry.
Oregon Green® 488	A10476	496	524	Fluorescent label for use in imaging or flow cytometry; hapten for signal amplification with anti-fluorescein/Oregon Green® antibodies.
Pacific Blue™ dye	A10478	416	451	Fluorescent label for use in imaging or flow cytometry.

Caution

DMSO (Component D), provided as a solvent in this kit, is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations.

Spectral Characteristics and Applications

For the best results in experiments, it is important to match the light source, excitation filters, and emission filters to the spectral characteristics of the dye. Refer to Table 2 for details.

Experimental Protocols

Antibody Labeling Procedure

Add buffers to the upper surface of the resin in the APEX antibody labeling tip using a gel-loading tip to minimize gel disturbance and introduction of air into the column (Figure 2A). After applying a buffer to the top of the resin, attach the APEX antibody labeling tip to a 200 µL-pipette (Figure 2B) and push the buffer into the resin until the top level of the buffer just enters the top of the resin bed. When pushing a buffer into or through the APEX antibody labeling tip, do not introduce air into the resin bed.

1.1 Gently tap the APEX antibody labeling tip (Component B) on a hard surface to settle all resin

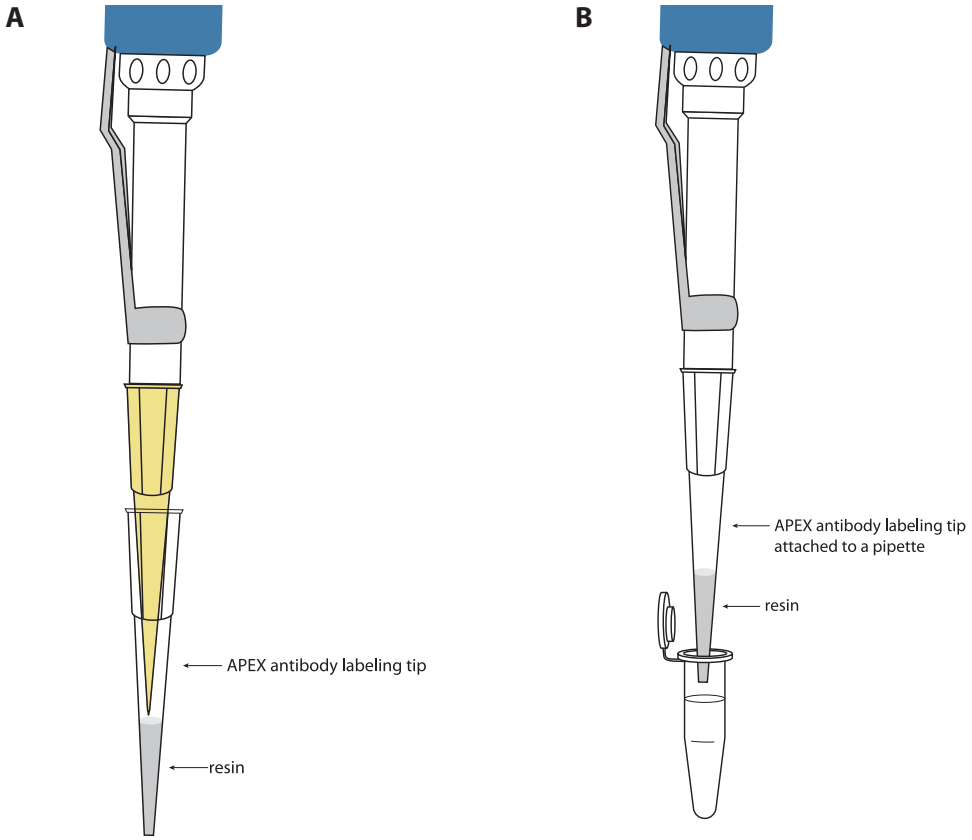

Figure 2. Using the APEX antibody labeling tip. Panel A: Applying solutions to the resin in the tip. Panel B: Pushing solutions onto the resin in the tip by attaching the APEX antibody labeling tip to a pipette.

at the bottom of the tip.

1.2 Remove both caps from the APEX antibody labeling tip and place the labeling tip into a clean microcentrifuge tube.

1.3 Hydrate the APEX antibody labeling tip (Component B) by applying 100 µL of wash buffer (Component C) to the resin in the APEX antibody labeling tip (Figure 2A) with a gel-loading tip. **Note:** Remove the pipette tip before releasing the plunger to ensure that the resin bed is not disturbed. Apply the APEX antibody labeling tip directly to the pipette and gently push the wash buffer through the tip into the microcentrifuge tube (Figure 2B).

The hydrated resin bed volume is 10–15 µL.

1.4 Apply 10–20 µg of IgG antibody solution to the top of the resin in the APEX antibody labeling tip, then gently push the antibody solution onto the resin. The antibody volume should not exceed 10 µL. After the antibody solution is pushed onto the column a drop may elute from the tip. Discard this eluent as waste.

1.5 To the vial of reactive dye (Component A), add the following:

- 2 µL DMSO (Component D); pipet up and down to dissolve
- 18 µL Labeling buffer (Component E); pipet up and down to dissolve

1.6 Apply 10 µL of the reactive dye from step 1.5 to the top of the resin, then gently push the solution onto the tip. A small amount of dye may elute from the tip. Discard this eluent as waste.

1.7 Incubate the tip for 2 hours at room temperature or overnight at 4°C.

1.8 Wash the APEX antibody labeling tip twice with 50 µL each with wash buffer (Component C) by applying 50 µL to the top of the resin, then pushing through the tip into the microcentrifuge tube.

1.9 To a **clean** microcentrifuge tube, add 10 µL neutralization buffer (Component F).

1.10 Position the APEX antibody labeling tip on the microcentrifuge tube containing the neutralization buffer and apply 40 µL elution buffer (Component G) to the top of the resin. Push through the tip to elute the labeled antibody into the microcentrifuge tube containing neutralization buffer.

Note: The ratio of neutralization buffer to elution buffer must remain 1:4 to ensure the correct pH. The elution can be performed with 20–40 µL elution buffer. Reducing the elution volume may increase the antibody concentration but result in a reduced total antibody yield.

1.11 Mix the labeled antibody solution to ensure neutralization. The final eluate volume is ~50 µL.

Cap the microcentrifuge tube containing the labeled antibody solution and place the tube on ice until use. The labeled antibody is ready for use in your imaging or flow cytometry application or store the antibody (see below).

Discard the APEX antibody labeling tip as biohazardous waste. **Do not** reuse the APEX antibody labeling tip.

Labeled Antibody Storage

The labeled antibody solution can be stored in the elution/neutralization buffer at 4°C for short-term storage (up to 2 weeks). For long-term storage, exchange the storage buffer with PBS or equivalent buffer by dialysis or gel filtration and store at –20°C. You may add other stabilization agents such as BSA to the labeled antibody solution, if desired.

Labeling Kits Invitrogen offers several other antibody and protein labeling kits optimized for labeling of smaller amounts of IgG antibody, or larger amounts of IgG antibody or proteins >30 kDa (Table 3).

Table 3. Antibody and protein labeling kits from Invitrogen.

IgG amount	Product	Features
<1–20 µg	Zenon® IgG Labeling Kit	• Labeled antibodies ready to use in 10 minutes • Isotype-specific labeling • Fast, noncovalent attachment of label • Labeling compatible with stabilizing proteins such as BSA
10–20 µg	APEX Antibody Labeling Kit	• Labeled antibodies ready to use in 2.5 hours (~15 minutes hands on time) • Covalent attachment of label • Labeling compatible with stabilizing proteins such as BSA
20–100 µg	Microscale Protein Labeling Kit	• Labeled antibodies ready to use in 2 hours (~30 minutes hands on time) • Covalent attachment of label • Optimized for proteins between 10–150 kDa, including IgG antibodies (~150 kDa) • Stabilizing proteins must be removed from sample before labeling
100 µg	Monoclonal Antibody Labeling Kit	• Labeled antibodies ready to use in 90 minutes (~15 minutes hands on time) • Covalent attachment of label • Optimized for IgG antibodies (~150 kDa) • Stabilizing proteins must be removed from sample before labeling • Designed to label polyclonal and monoclonal IgG antibodies
1 mg	Protein Labeling Kit	• Labeled antibodies ready-to-use in 2 hours (~30 minutes hands on time) • Covalent attachment of label • Optimized for IgG antibodies (~150 kDa) • Stabilizing proteins must be removed from sample before labeling

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
A10468	APEX Alexa Fluor® 488 Antibody Labeling Kit	1 kit
A10470	APEX Alexa Fluor® 555 Antibody Labeling Kit	1 kit
A10474	APEX Alexa Fluor® 594 Antibody Labeling Kit	1 kit
A10475	APEX Alexa Fluor® 647 Antibody Labeling Kit	1 kit
A10476	APEX Oregon Green® 488 Antibody Labeling Kit	1 kit
A10478	APEX Pacific Blue™ Antibody Labeling Kit	1 kit
Related Products		
A11094	anti-Alexa Fluor® 488, rabbit IgG fraction *1 mg/mL*	0.5 mL
A889	anti-fluorescein/Oregon Green®, rabbit IgG fraction *1 mg/mL*	0.5 mL
A982	anti-fluorescein/Oregon Green®, rabbit IgG fraction, biotin-XX conjugate *1 mg/mL*	0.5 mL
A6413	anti-fluorescein/Oregon Green®, rabbit IgG Fab fragment *0.5 mg/mL*	0.5 mL
A6421	anti-fluorescein/Oregon Green®, mouse IgG_{2a}, monoclonal 4-4-20	0.5 mg
A11090	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A11091	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 594 conjugate *1 mg/mL*	0.5 mL
A11095	anti-fluorescein/Oregon Green®, goat IgG fraction *1 mg/mL*	0.5 mL
A11096	anti-fluorescein/Oregon Green®, goat IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A21250	anti-fluorescein/Oregon Green®, rabbit IgG fraction, R-phycoerythrin conjugate *2 mg/mL*	250 µL
A21253	anti-fluorescein/Oregon Green®, rabbit IgG fraction, horseradish peroxidase conjugate	0.5 mg
Q10137MP	Qdot® 565 goat anti-fluorescein conjugate *2 µM* *whole IgG*	500 µL
Q15421MP	Qdot® 565 goat anti-fluorescein conjugate *2 µM* *whole IgG*	200 µL

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