

Zenon[®] Mouse IgG Labeling Kits

Table 1 Contents and storage

Material	Amount	Concentration*	Storage†
Zenon[®] Mouse IgG Labeling Kits for labeling with a low molecular weight dye or biotin Sufficient reagents are provided for 50 labelings.			
Zenon [®] mouse IgG labeling reagent (Component A)	250 µL	200 µg Fab fragment/mL	<ul style="list-style-type: none"> • 2–8°C • Protect from light
Zenon [®] blocking reagent (mouse IgG) (Component B)	250 µL	5 mg/mL	
Zenon[®] Mouse IgG Labeling Kits for labeling with R-phycoerythrin (R-PE), allophycocyanin (APC) Sufficient reagents are provided for 25 labelings.			
Zenon [®] mouse IgG labeling reagent (Component A)	125 µL	200 µg Fab fragment/mL	<ul style="list-style-type: none"> • 2–8°C • Protect from light • DO NOT FREEZE
Zenon [®] blocking reagent (mouse IgG) (Component B)	125 µL	5 mg/mL	
Zenon[®] Mouse IgG Labeling Kits for labeling with horseradish peroxidase Sufficient reagents are provided for 25 labelings.			
Zenon [®] mouse IgG labeling reagent (Component A)	125 µL	200 µg Fab fragment/mL	<ul style="list-style-type: none"> • 2–8°C • Protect from light • DO NOT FREEZE
Zenon [®] blocking reagent (mouse IgG) (Component B)	125 µL	5 mg/mL	
Zenon[®] Mouse IgG Labeling Kits for labeling with Alexa Fluor[®] dye–R-PE or Alexa Fluor[®] dye–APC tandem constructs Sufficient reagents are provided for 10 labelings.			
Zenon [®] mouse IgG labeling reagent (Component A)	50 µL	200 µg Fab fragment/mL	<ul style="list-style-type: none"> • 2–8°C • Protect from light • DO NOT FREEZE
Zenon [®] blocking reagent (mouse IgG) (Component B)	50 µL	5 mg/mL	
Zenon[®] Tricolor Mouse IgG Labeling Kits (see Table 3) Sufficient reagents are provided for 10 labelings with each of the three included labeling reagents.			
Zenon [®] mouse IgG labeling reagent of one fluorescent color (Component A)	50 µL	200 µg Fab fragment/mL	<ul style="list-style-type: none"> • 2–8°C • Protect from light
Zenon [®] mouse IgG labeling reagent of a second fluorescent color (Component B)	50 µL	200 µg Fab fragment/mL	
Zenon [®] mouse IgG labeling reagent of a third fluorescent color (Component C)	50 µL	200 µg Fab fragment/mL	
Zenon [®] blocking reagent (mouse IgG) (Component D)	50 µL	5 mg/mL	
* See Kit Contents , page 2, for more information. † For long-term storage, Zenon [®] mouse IgG labeling reagents containing low molecular weight fluorophores or biotin and the Zenon [®] blocking reagent can be divided into single-use aliquots and frozen at ≤–20°C. Do not freeze Zenon [®] mouse IgG labeling reagents containing R-phycoerythrin, allophycocyanin, Alexa Fluor [®] dye–R-PE or Alexa Fluor [®] dye–APC tandem constructs, or horseradish peroxidase. When stored as directed, the kits are stable for 6 months after receipt.			
Spectral data: See Table 2 (page 4).			

The Zenon[®] Mouse IgG Labeling Kits provide a fast, versatile and reliable method for producing antibody conjugates, even with very small (submicrogram) amounts of starting material. Antibody conjugates formed using Zenon[®] technology may be used to stain cells in any application where a directly labeled primary antibody is suitable, including flow cytometry, imaging, high throughput and other applications. Moreover, this technology simplifies applications that previously were time consuming or not practical, such as the use of multiple mouse-derived antibodies in the same staining protocol.

Each of the Zenon[®] Mouse IgG Labeling Kits is designed for use with a particular mouse monoclonal antibody isotype: IgG₁, IgG_{2a} or IgG_{2b}. The available labels consist of a large selection of premium fluorescent dyes, including our entire range of Alexa Fluor[®] dyes, as well as conventional dyes, R-phycoerythrin (R-PE), allophycocyanin (APC), Alexa Fluor[®] dye-R-PE or Alexa Fluor[®] dye-APC tandem constructs, enzymes and biotin (Table 2, page 4). We also offer Zenon[®] Tricolor Mouse IgG Labeling Kits, each with a selection of three different Zenon[®] mouse IgG labeling reagents (Table 3, page 4).

Zenon[®] labeling technology utilizes a fluorophore-, biotin- or enzyme-labeled Fab fragment directed against the Fc portion of an intact IgG primary antibody in order to form a labeling complex (Figure 1, page 3). The labeled Fab fragments have been affinity purified during their preparation to ensure their high affinity and selectivity for the Fc portion of the primary antibody. Because this labeling is based on immunoselectivity, the Zenon[®] labeling method does not require the removal of exogenous proteins such as serum albumin or amine-containing buffers from the antibody prior to forming the complex. Crossreactivity is low with antibodies from other species.

Formation of the Fab-antibody complex occurs in less than 5 minutes, and nearly all of the primary antibodies in the mixture are labeled (Figure 2, page 3). Complexes formed using this technology display fluorescence intensity or enzymatic activity similar to that of directly labeled primary antibodies. In addition, the extent of antibody labeling (and thus the fluorescence intensity or enzymatic activity of the probe) can be adjusted by varying the amount of Zenon[®] labeling reagent that is added, i.e. by varying the molar ratio of labeled Fab fragment to primary antibody.

Kit Contents

Zenon[®] mouse IgG labeling reagents are labeled goat Fab fragments selective for the Fc portion of mouse IgG antibodies of the indicated IgG isotype—IgG₁, IgG_{2a} or IgG_{2b}—depending on the kit.

- Zenon[®] mouse IgG labeling reagents conjugated to a low molecular weight dye or biotin are supplied at 200 µg Fab fragment/mL in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide, except the Zenon[®] Pacific Orange[™] mouse IgG labeling reagents, which are provided at a concentration of 400 µg/mL.
- Zenon[®] mouse IgG labeling reagents conjugated to R-phycoerythrin, allophycocyanin, Alexa Fluor[®] dye-R-PE or Alexa Fluor[®] dye-APC tandem constructs are supplied at 200 µg Fab fragment/mL in 0.1 M sodium phosphate, 0.1 M NaCl, pH 6.8, containing 5 mM sodium azide.
- Zenon[®] mouse IgG labeling reagents conjugated to horseradish peroxidase are supplied at 200 µg Fab fragment/mL in 0.1 M sodium phosphate, 0.1 M NaCl, pH 6.8, containing 0.02% thimerosal.
- The Zenon[®] blocking reagent (mouse IgG) is supplied at 5 mg/mL in phosphate-buffered saline, pH 7.2, containing 5 mM sodium azide.

The Zenon[®] Mouse IgG Labeling Kits contain sufficient reagents for 50, 25, or 10 labelings, depending upon the kit (see Table 1, page 1). One labeling is defined as the amount of Zenon[®] labeling reagent required to label 1 µg of an intact, affinity-purified mouse IgG antibody at a Fab:antibody molar ratio of 3:1 (note A, page 9).

Figure 1 The Zenon® labeling scheme. **(A)** An unlabeled IgG is incubated with the Zenon® labeling reagent, which contains a fluorophore-labeled Fab fragment. **(B)** The labeled Fab fragment binds to the Fc portion of the IgG antibody. **(C)** Excess Fab fragment is neutralized 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 need not be coupled to a fluorophore, but could instead be coupled to an enzyme or to biotin.

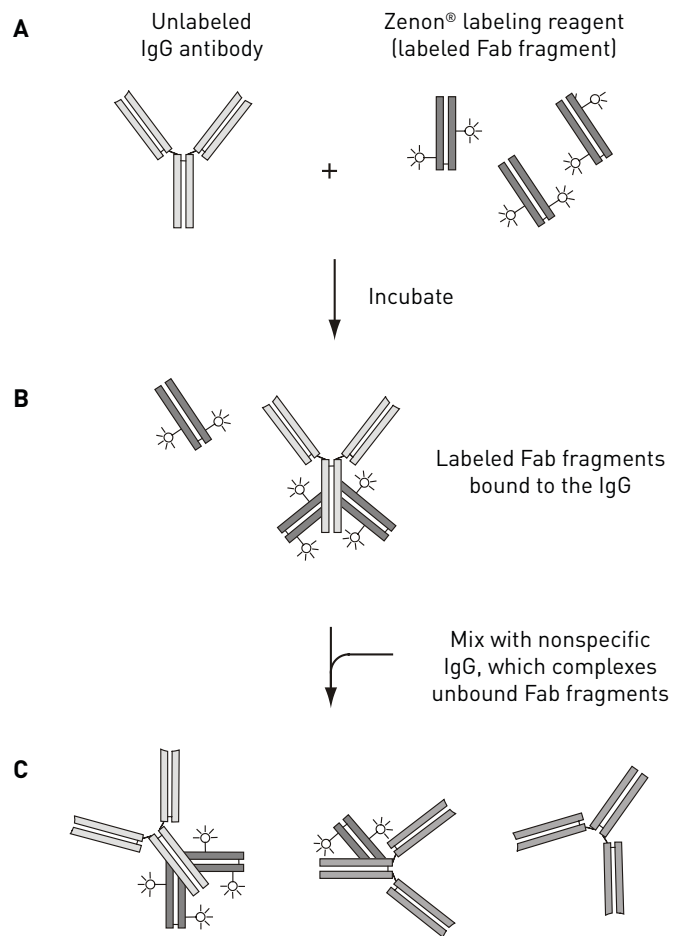


Figure 2 High-performance size-exclusion chromatographic analysis of the Alexa Fluor® 488 Zenon® labeling reagent binding to a mouse IgG₁ antibody. The Zenon® labeling reagent, alone, appears as a peak at 38 minutes; the mouse IgG₁, alone, at 33 minutes. When mixed together at a molar ratio of ~5:1 (Zenon® labeling reagent to IgG₁), the IgG₁ is quantitatively converted to a labeled complex that appears as a peak at 29 minutes.

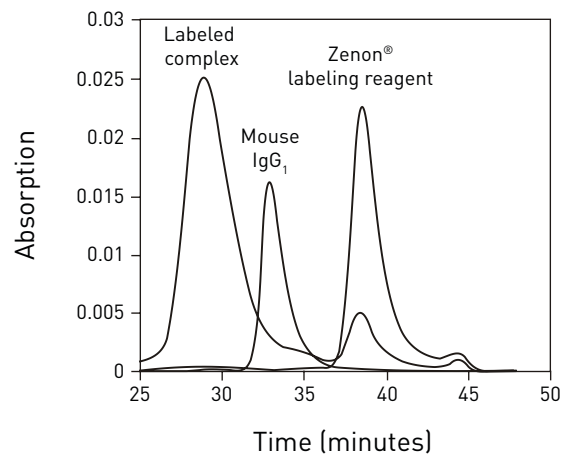


Table 2 Zenon® Mouse IgG Labeling Kits available from Life Technologies

Label	Ex/Em *	Catalog Number		
		Mouse IgG ₁	Mouse IgG _{2a}	Mouse IgG _{2b}
Alexa Fluor® dyes				
Alexa Fluor® 350	346/442	Z25000	Z25100	Z25200
Alexa Fluor® 405	402/421	Z25013	Z25113	Z25213
Alexa Fluor® 488	495/519	Z25002	Z25102	Z25202
Alexa Fluor® 532	531/554	Z25003		
Alexa Fluor® 546	556/573	Z25004	Z25104	Z25204
Alexa Fluor® 555	555/565	Z25005	Z25105	Z25205
Alexa Fluor® 568	578/603	Z25006	Z25106	Z25206
Alexa Fluor® 594	590/617	Z25007	Z25107	Z25207
Alexa Fluor® 647	650/668	Z25008	Z25108	Z25208
Alexa Fluor® 660	663/690	Z25009		
Alexa Fluor® 680	679/702	Z25010	Z25110	Z25210
Alexa Fluor® 700	696/719	Z25011		
Conventional dyes				
Pacific Orange™	400/551	Z25256	Z25257	
Pacific Blue™	410/455	Z25041	Z25156	
Pacific Green™	411/500	Z11203		
Fluorescein	494/518	Z25042		
Texas Red®-X	595/615	Z25045		
Biotins				
Biotin-XX	NA	Z25052	Z25152	Z25252
R-phycoerythrin, allophycocyanin and tandem constructs				
R-phycoerythrin (R-PE)	496/578 †	Z25055	Z25155	Z25255
Alexa Fluor® 610-R-PE	496/630 †	Z25020		
Alexa Fluor® 647-R-PE	496/668 †	Z25021		Z25221
Alexa Fluor® 680-R-PE	496/702 †	Z25022		
Allophycocyanin (APC)	650/660	Z25051	Z25151	Z25251
Alexa Fluor® 750-APC	650/775	Z25031		
Enzymes				
Horseradish peroxidase	NA	Z25054	Z25154	Z25254
* Approximate fluorescence excitation and emission maxima, in nm. † Additional absorption peaks are present at 546 and 565 nm. NA = not applicable.				

Table 3 Zenon® Tricolor Mouse IgG Labeling Kits available from Life Technologies

Tricolor Labeling Kit	Labels *	Catalog Number		
		Mouse IgG ₁	Mouse IgG _{2a}	Mouse IgG _{2b}
Kit #1 (for imaging)	Alexa Fluor® 488 Alexa Fluor® 555 Alexa Fluor® 647	Z25060	Z25160	Z25260
Kit #2 (for imaging)	Alexa Fluor® 350 Alexa Fluor® 488 Alexa Fluor® 594	Z25070	Z25170	
Kit #3 (for flow cytometry)	Alexa Fluor® 488 R-phycoerythrin (R-PE) Alexa Fluor® 647-R-PE	Z25080	Z25180	
* See Table 2, above, for fluorescence excitation and emission maxima.				

Zenon® Complex Formation

The following protocol is for labeling 1 µg of antibody with a Zenon® mouse IgG labeling reagent to obtain a 3:1 molar ratio of Fab to antibody target (note A, page 9). This molar ratio is a suggested starting point and represents the minimum ratio for adequate labeling in most applications; individual experiments may require higher molar ratios in order to obtain satisfactory signal (note B, page 9). For larger or smaller quantities of antibody, the amounts of the reagents specified in this protocol can be scaled accordingly. The Zenon® mouse IgG labeling reaction does not require the removal of bovine serum albumin (BSA) or other stabilizing proteins that may be present in antibody preparations. Antibodies contained within ascites fluid or hybridoma supernatants can also be directly labeled and do not require purification of the antibody either prior to or after labeling.

NOTE: Before beginning the labeling protocol, verify that the isotype of the mouse antibody matches that of the Zenon® Mouse IgG Labeling Kit. The Zenon® mouse IgG labeling reagents in the kits are isotype specific and are not recommended for labeling antibodies that are not of the corresponding mouse IgG isotype.

- 1.1 Prepare 1 µg of antibody in a suitable buffer, such as phosphate-buffered saline (PBS). The volume is not crucial, provided it is ≤20 µL. If using an antibody where the concentration is not specified, refer to note C, page 10. If using an impure antibody preparation, e.g., ascites fluid or hybridoma supernatant, refer to note D, page 10.
- 1.2 Add 5 µL of the Zenon® mouse IgG labeling reagent (Component A) to the antibody solution. (If using a Zenon® Tricolor Kit, use the desired labeling reagent from Component A, B, or C.)
- 1.3 Incubate the mixture for 5 minutes at room temperature.
- 1.4 Add 5 µL of the Zenon® blocking reagent (Component B, or Component D if using a Zenon® Tricolor Kit) to the reaction mixture. If using a Zenon® Pacific Orange™ labeling kit, add 10 µL of the Zenon® blocking reagent. The blocking reagent is not isotype specific and is the same in all Zenon® Mouse IgG Labeling Kits. If the application uses only a single labeled antibody, refer to note E, page 10.
- 1.5 Incubate the solution for 5 minutes at room temperature.

The complexes are now ready and should be applied to samples within approximately 30 minutes. If longer storage is required, refer to note F, page 10.

General Application Tips

Conjugate utility. Mouse IgG antibodies labeled using Zenon® technology are expected to be suitable in all applications where a directly labeled antibody can be used. Multiple mouse IgG antibodies labeled with Zenon® reagents can be used in one experiment either sequentially or as a single staining mixture.

Conjugate stability. Once the conjugates have been formed and excess Fab taken up by the blocking reagent, the labeled complexes should be used within approximately 30 minutes.

Working concentration. For applications where a directly labeled primary antibody is typically used, the antibody labeled using Zenon® technology can generally be used at a similar or higher concentration (1.5–3-fold).

Low or no signal. If suitable controls verify that the primary antibody is binding to the expected target but no signal is observed with the antibody labeled using Zenon[®] technology, the signal may be increased by adding more of the Zenon[®] labeling reagent to increase the Fab:antibody molar ratio and/or by increasing the final concentration of the primary antibody. While a molar ratio of 3:1 is suitable in many cases, the molar ratio can be increased up to 6:1. Further increases in the molar ratio will not result in a significant increase in the signal strength.

Label loss or transfer. Because the labeled Fab fragment is not covalently coupled to the primary antibody, some loss of the labeled Fab fragments may occur over time. In applications with multiple mouse IgG primary antibodies, the Fab fragments may transfer slowly from one primary antibody to another. Samples should be subjected to an additional post-staining fixation step with formaldehyde in order to reduce Fab dissociation (see **Staining Applications**, below).

Staining Applications

The following protocols were developed at Life Technologies for the staining of cells and tissues using primary antibodies labeled with Zenon[®] mouse IgG labeling reagents. The protocols may not be applicable to all experimental situations, and users are encouraged to adapt these protocols to their needs.

Immunocytochemistry

This protocol has been successfully used to stain a number of cell lines, including bovine pulmonary artery endothelial (BPAE), Muntjac and Madin-Darby canine kidney (MDCK) cells. For primary antibodies against abundant targets, forming Zenon[®] labeling complexes at a molar ratio of 3:1, Zenon[®] labeling reagent (Fab) to antibody, is suitable; for less abundant targets, a molar ratio of 6:1 is recommended.

- 2.1 Culture cells in suitable growth medium. Adherent cell lines intended for observation by microscopy can be grown directly on slides or coverslips; however, do not allow the slides or coverslips to dry out at any point during the staining protocol.
- 2.2 Fix the cell sample in 4% formaldehyde solution in PBS for 15 minutes at room temperature. Alternatively, the sample can be incubated for 15 minutes at 37°C under 5% CO₂. This latter fixation method has been observed to better preserve cytoskeletal morphology.
- 2.3 Wash the cell sample one or more times with PBS.
- 2.4 Permeabilize the cells by incubating the sample in PBS containing 0.1% Triton[®] X-100 for 5 minutes at room temperature. Remove the detergent solution.
- 2.5 Block nonspecific binding sites in the cell sample for 30 minutes in PBS containing 10% normal goat serum (NGS).
- 2.6 Dilute the Zenon[®] labeling complex (prepared according to **Zenon[®] Complex Formation**, page 5) to the desired working concentration in PBS containing 10% NGS, and apply a sufficient volume to immerse the cell sample. If staining with more than one Zenon[®] labeling complex, all complexes can be combined into a single staining solution; sequential labeling is not necessary.

Note: In systems where the concentration of primary antibody has already been optimized for use with a secondary detection reagent, use the primary antibody at a concentration 1.5–3-fold higher with the Zenon[®] staining method. Otherwise, the appropriate working concentration will need to be determined empirically.

2.7 Incubate the cell sample for 30–60 minutes at room temperature. Zenon® labeling reagents with fluorophores should be protected from light during the incubation. When staining is complete, wash the cell sample one or more times with PBS.

2.8 Perform a second fixation of the cell sample in 4% formaldehyde solution in PBS for 15 minutes at room temperature. When the fixation is complete, wash the cell sample one or more times with PBS.

Note: Without this second fixation step, the Zenon® Fab fragment, which is not covalently coupled to the primary antibody, can dissociate with time and can even transfer to another primary antibody in multicolor applications. This step is optional for experiments using only a single primary antibody for labeling purposes, but signal intensity is generally superior with the additional fixation.

2.9 If desired, counterstain the cell sample with a nucleic acid stain (e.g., Hoechst 33342, DAPI, or SYTOX® Green stains) or with other labeling reagents (e.g., fluorophore-labeled phalloidin), and wash with PBS after any additional staining procedure.

2.10 If necessary, deposit the cells on a slide or coverslip, or place in a flow tube. For a sample to be viewed by microscopy, mount in a suitable antifade mounting medium (e.g., use the ProLong® Gold antifade reagent; Cat. no. P36930). Analyze or view the sample by the desired method.

Immunohistochemistry

This protocol is designed for 10–12 µm frozen sections of tissues fixed in 4% formaldehyde via transcardial perfusion. **Do not use glutaraldehyde-containing fixatives.** The use of glutar-aldehyde increases sample autofluorescence and results in excessive crosslinking of proteins, which can impede antibody access and can denature antigens. Where possible, use thinner sections to promote thorough and more rapid antibody penetration into the tissue. Zenon® labeling complexes have not been tested or optimized for sections embedded in paraffin. Note that certain low-abundance antigens may not be detected as easily using Zenon® labeling technology as they use conventional secondary detection methods, and the Zenon® method is not recommended for very low-abundance target molecules in tissue samples.

In parallel to the labeling of an experimental sample, a negative-control sample should be processed in an identical protocol, but with the omission of the primary antibody, in order to assess nonspecific labeling. A positive control sample labeled by conventional secondary detection methods can be included during pilot studies to confirm the specificity of the staining pattern observed using the Zenon® labeling method.

3.1 Allow the slide containing the frozen tissue section to thaw at room temperature (usually 15–30 minutes). Draw a rectangle around the section with a PAP pen, if desired.

3.2 Rehydrate the section in PBS for 15 minutes at room temperature. Do not allow the section to dry at any point during the remaining steps of this staining protocol.

3.3 Permeabilize the section in PBS containing 0.2% Triton® X-100 (PBST) for 20 minutes at room temperature.

3.4 Block nonspecific binding sites with PBST containing 0.2% BSA and 5% heat-inactivated normal goat serum for 30 minutes at room temperature. Alternatively, 1% BSA can be used in place of the serum. Remove the blocking buffer once the incubation is complete.

- 3.5 Dilute the Zenon[®] labeling complex (prepared according to **Zenon[®] Complex Formation**, page 5) in PBST to the desired working concentration. In a horizontal staining dish, apply 100–250 µL of the working solution to cover the section.

Note: For initial experiments, test a range of different primary antibody concentrations and different molar ratios of Zenon[®] labeling reagent (Fab) to antibody. In general, Zenon[®] labeling methods require a higher concentration of primary antibody than would be used in a standard secondary detection scheme. A molar ratio of 6:1 is the recommended starting point for tissue staining. It is important to include detergent in the staining buffer to promote antibody penetration. Buffer PBST, as described above, contains 0.2% Triton[®] X-100; however, the detergent choice and concentration (generally 0.1–0.5%) may need to be optimized to strike a balance between tissue permeabilization and the possibility of the antigen being sensitive to detergent extraction.

- 3.6 Incubate the section with the staining solution in a humidified chamber for 1–2 hours at room temperature. Zenon[®] labeling reagents with fluorophores should be protected from light during the incubation. If staining with more than one Zenon[®] labeling complex, all complexes can be combined into a single staining solution; sequential labeling is not necessary. **Do not use longer incubation times.**

Note: With longer incubation times, the Zenon[®] Fab fragment can dissociate from the primary antibody, resulting in poor signal-to-noise ratios and possible crosslabeling of primary antibodies in multicolor staining applications.

- 3.7 Remove the staining solution and wash the section in PBST for 10–15 minutes at room temperature. Repeat the wash an additional two times. The wash steps should be performed in an upright Coplin staining jar.

- 3.8 Wash the stained tissue section for 5 minutes in PBS at room temperature. Repeat.

- 3.9 Perform a second fixation of the tissue section in 4% formaldehyde solution in PBS for 15 minutes at room temperature. When the fixation is complete, wash the section one or more times with PBS.

Note: Without this second fixation step, the Zenon[®] Fab fragment, which is not covalently coupled to the primary antibody, can dissociate with time, and can even transfer to another primary antibody in multicolor applications.

- 3.10 If desired, counterstain the tissue sample with a nucleic acid stain (e.g., Hoechst 33342, DAPI, or SYTOX[®] Green stains) or with other labeling reagents (e.g., fluorophore-labeled phalloidin), and wash with PBS after any additional staining procedure.

- 3.11 Mount the section in a suitable antifade mounting medium (e.g., use the ProLong[®] antifade reagent; Cat. no. P36930) and observe the fluorescence using appropriate filters.

Flow Cytometry

The following protocol was developed for the staining of peripheral blood mononuclear cells and can be readily adapted to other cell types or to whole blood samples. For primary antibodies against abundant targets, forming Zenon[®] labeling complexes at a molar ratio of 3:1, Zenon[®] labeling reagent (Fab) to antibody, is suitable; for less abundant targets the molar ratio should be increased. Note that an Fc blocking step is not required when using antibodies labeled with Zenon[®] technology.

- 4.1 Prepare the cell suspension or blood sample as preferred.

- 4.2 Add 100 μL of cultured cells (at 1×10^6 cells/mL) or 100 μL of a whole blood sample to a flow cytometry tube.
- 4.3 *Optional:* Fix the cell sample in 4% formaldehyde solution in PBS for 15 minutes at room temperature. Wash the cell sample with PBS, centrifuge the sample to pellet the cells and decant the supernatant. Resuspend the cells in the residual buffer.

Note: This and the following step are required only when detecting internal cell markers. If using antibodies against cell surface and internal markers in the same experiment, perform the staining of the cell surface markers first, then follow this step and steps 4.4–4.7 to stain the internal markers.

- 4.4 *Optional:* Permeabilize the cell sample by incubating the sample in PBS containing 0.1% Triton[®] X-100 or other permeabilization reagent for 5 minutes at room temperature. Wash the cell sample with PBS, centrifuge the sample to pellet the cells and decant the supernatant. Resuspend the cells in the residual buffer.
- 4.5 Label 1 μg of the primary antibody according to Zenon[®] Complex Formation, above.
- 4.6 Add the entire Zenon[®] labeling mixture (prepared in step 4.5) to the sample tube.

Note: If using whole blood samples, the lysis of red blood cells can be performed either before or after cell staining (steps 4.6–4.7).

- 4.7 Incubate the sample for 30 minutes at room temperature. Zenon[®] labeling complexes with fluorophores should be protected from light during the incubation. When complete, wash the cells once with PBS, centrifuge the sample to pellet the cells, and decant the supernatant. Resuspend the cells in the residual buffer. A post-staining fixation step, required for imaging applications, is not required for flow cytometry applications.
- 4.8 Analyze the sample using appropriate instrument parameters.

Product Notes

[A] The Fab:antibody ratio is the important factor when determining the amount of the Zenon[®] mouse IgG labeling reagent to use in the labeling protocol. In all Zenon[®] Mouse IgG Labeling Kits except Zenon[®] Pacific Orange[™] Mouse IgG Labeling Kits, the Zenon[®] labeling reagent is provided at a concentration of 200 $\mu\text{g}/\text{mL}$ **based on the mass of the Fab fragment**. A Fab fragment has a molecular weight of ~ 50 kDa, compared to ~ 150 kDa for an intact IgG; thus, 5 μL of any Zenon[®] labeling reagent (except Zenon[®] Pacific Orange[™] labeling reagent) mixed with 1 μg of mouse IgG antibody produces a Fab:antibody molar ratio of 3:1.

In Zenon[®] Pacific Orange[™] Mouse IgG labeling kits, the Zenon[®] labeling reagent is provided at a concentration of 400 $\mu\text{g}/\text{mL}$ **based on the mass of the Fab fragment**. Thus, 5 μL of any Zenon[®] Pacific Orange[™] labeling reagent mixed with 1 μg of mouse IgG antibody produces a Fab:antibody molar ratio of 6:1.

[B] When adjusting either the amount of antibody to be labeled or the Fab:antibody molar ratio, it is important to always use equal volumes of Zenon[®] labeling reagent and Zenon[®] blocking reagent. For example, if the amount of Zenon[®] labeling reagent used for a reaction is increased to 10 μL , then the amount of Zenon[®] blocking reagent should also be increased to 10 μL . Note that adding 10 μL of the Zenon[®] labeling reagent for each microgram of antibody (yielding a molar ratio of 6:1) will often increase the measured signal intensities by approximately 50%. Further increases in the molar ratio tend to yield smaller increases in intensity.

- [C] If the concentration of mouse IgG is not specified, contact the supplier and obtain at least an approximate IgG content. If a concentration cannot be obtained from the supplier, a titration with the Zenon® labeling reagent will need to be performed to obtain the optimum labeling conditions.
- [D] Monoclonal antibodies from suppliers are generally provided as a purified IgG fraction, as ascites fluid or as hybridoma supernatant. Primary antibodies that have not been purified can still be labeled using the Zenon® mouse IgG labeling reagents and do not require the removal of nonspecific IgGs or serum proteins. The appropriate amount of the Zenon® labeling reagent to add in step 1.2 should be determined by using the total IgG mass in the sample to be labeled; thus, 5 µL of the Zenon® mouse IgG labeling reagent should be used for each µg of IgG. Nonspecific IgGs will be labeled in addition to the specific IgG; however, the labeled nonspecific IgGs should not stain the sample appreciably and will be washed away during the staining procedure.
- [E] If mouse cells or tissues are not being used, and if the application requires only a single labeled antibody, steps 1.4 and 1.5 of the labeling protocol (addition of the blocking reagent) can be omitted. The level of uncomplexed Zenon® labeling reagent is sufficiently low that it should not cause high background levels.
- [F] If long-term storage of the labeling complexes is desired, stop the labeling procedure at step 1.3 of the protocol. At this stage the complexes can be stored at 4°C for several weeks with the addition of 2 mM sodium azide as a preservative. When ready to use the conjugate, complete steps 1.4 and 1.5 of the protocol before applying the conjugate to the experimental sample.

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

Cat. no.	Product Name	Unit Size
Z11203	Zenon® Pacific Green™ Mouse IgG ₁ Labeling Kit	1 kit
Z25000	Zenon® Alexa Fluor® 350 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25001	Zenon® Alexa Fluor® 430 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25002	Zenon® Alexa Fluor® 488 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25003	Zenon® Alexa Fluor® 532 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25004	Zenon® Alexa Fluor® 546 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25005	Zenon® Alexa Fluor® 555 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25006	Zenon® Alexa Fluor® 568 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25007	Zenon® Alexa Fluor® 594 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25008	Zenon® Alexa Fluor® 647 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25009	Zenon® Alexa Fluor® 660 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25010	Zenon® Alexa Fluor® 680 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25011	Zenon® Alexa Fluor® 700 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25013	Zenon® Alexa Fluor® 405 Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25020	Zenon® Alexa Fluor® 610-R-Phycoerythrin Mouse IgG ₁ Labeling Kit *10 labelings*	1 kit
Z25021	Zenon® Alexa Fluor® 647-R-Phycoerythrin Mouse IgG ₁ Labeling Kit *10 labelings*	1 kit
Z25022	Zenon® Alexa Fluor® 680-R-Phycoerythrin Mouse IgG ₁ Labeling Kit *10 labelings*	1 kit
Z25030	Zenon® Alexa Fluor® 700-Allphycocyanin Mouse IgG ₁ Labeling Kit *10 labelings*	1 kit
Z25031	Zenon® Alexa Fluor® 750-Allphycocyanin Mouse IgG ₁ Labeling Kit *10 labelings*	1 kit
Z25041	Zenon® Pacific Blue™ Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25042	Zenon® Fluorescein Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25045	Zenon® Texas Red®-X Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25051	Zenon® Allophycocyanin Mouse IgG ₁ Labeling Kit *25 labelings*	1 kit
Z25052	Zenon® Biotin-XX Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25054	Zenon® Horseradish Peroxidase Mouse IgG ₁ Labeling Kit *25 labelings*	1 kit
Z25055	Zenon® R-Phycoerythrin Mouse IgG ₁ Labeling Kit *25 labelings*	1 kit
Z25060	Zenon® Tricolor Mouse IgG ₁ Labeling Kit #1 *for green, orange and deep red fluorescence imaging* *3 × 10 labelings*	1 kit

Z25070	Zenon® Tricolor Mouse IgG ₁ Labeling Kit #2 *for blue, green and red fluorescence imaging* *3 × 10 labelings*	1 kit
Z25080	Zenon® Tricolor Mouse IgG ₁ Labeling Kit #3 *for flow cytometry, 488 nm excitation* *3 × 10 labelings*	1 kit
Z25100	Zenon® Alexa Fluor® 350 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25102	Zenon® Alexa Fluor® 488 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25104	Zenon® Alexa Fluor® 546 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25105	Zenon® Alexa Fluor® 555 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25106	Zenon® Alexa Fluor® 568 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25107	Zenon® Alexa Fluor® 594 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25108	Zenon® Alexa Fluor® 647 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25110	Zenon® Alexa Fluor® 680 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25113	Zenon® Alexa Fluor® 405 Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25151	Zenon® Allophycocyanin Mouse IgG _{2a} Labeling Kit *25 labelings*	1 kit
Z25152	Zenon® Biotin-XX Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25154	Zenon® Horseradish Peroxidase Mouse IgG _{2a} Labeling Kit *25 labelings*	1 kit
Z25155	Zenon® R-Phycoerythrin Mouse IgG _{2a} Labeling Kit *25 labelings*	1 kit
Z25156	Zenon® Pacific Blue™ Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25160	Zenon® Tricolor Mouse IgG _{2a} Labeling Kit #1 *for green, orange and deep red fluorescence imaging* *3 × 10 labelings*	1 kit
Z25170	Zenon® Tricolor Mouse IgG _{2a} Labeling Kit #2 *for blue, green and red fluorescence imaging* *3 × 10 labelings*	1 kit
Z25180	Zenon® Tricolor Mouse IgG _{2a} Labeling Kit #3 *for flow cytometry, 488 nm excitation* *3 × 10 labelings*	1 kit
Z25200	Zenon® Alexa Fluor® 350 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25202	Zenon® Alexa Fluor® 488 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25204	Zenon® Alexa Fluor® 546 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25205	Zenon® Alexa Fluor® 555 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25206	Zenon® Alexa Fluor® 568 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25207	Zenon® Alexa Fluor® 594 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25208	Zenon® Alexa Fluor® 647 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25210	Zenon® Alexa Fluor® 680 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25213	Zenon® Alexa Fluor® 405 Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25221	Zenon® Alexa Fluor® 647-R-Phycoerythrin Mouse IgG _{2b} Labeling Kit *10 labelings*	1 kit
Z25251	Zenon® Allophycocyanin Mouse IgG _{2b} Labeling Kit *25 labelings*	1 kit
Z25252	Zenon® Biotin-XX Mouse IgG _{2b} Labeling Kit *50 labelings*	1 kit
Z25254	Zenon® Horseradish Peroxidase Mouse IgG _{2b} Labeling Kit *25 labelings*	1 kit
Z25255	Zenon® R-Phycoerythrin Mouse IgG _{2b} Labeling Kit *25 labelings*	1 kit
Z25256	Zenon® Pacific Orange™ Mouse IgG ₁ Labeling Kit *50 labelings*	1 kit
Z25257	Zenon® Pacific Orange™ Mouse IgG _{2a} Labeling Kit *50 labelings*	1 kit
Z25260	Zenon® Tricolor Mouse IgG _{2b} Labeling Kit #1 *for green, orange and deep red fluorescence imaging* *3 × 10 labelings*	1 kit
Z25270	Zenon® Tricolor Mouse IgG _{2b} Labeling Kit #2 *for blue, green and red fluorescence imaging* *3 × 10 labelings*	1 kit

Purchaser Notification

Corporate Headquarters

5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European Headquarters

Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese Headquarters

LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
Fax: +81 3 5730 6519
Email: jpinfo@invitrogen.com

Additional international offices are listed at
www.lifetechnologies.com

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining Support

For the latest services and support information for all locations, go to www.lifetechnologies.com.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners.

Cy3® and Cy5® are registered trademarks of GE Healthcare.

Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology Corp.

©2013 Life Technologies Corporation. All rights reserved.

