Zenon™ Mouse IgG Labeling Kits

Pub. No. MAN0025408 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ 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: IgG_1, IgG_{2a} or IgG_{2b}. The available labels consist of a large selection of premium fluorescent dyes, including our Alexa Fluor™ dyes, as well as conventional dyes, R-phycoerythrin (R-PE), allophycocyanin (APC) or dye–R-PE tandem constructs, horseradish peroxidase (HRP) and biotin. We also offer Zenon™ Tricolor Mouse IgG Labeling Kits, each with a selection of three different Zenon™ mouse IgG labeling reagents.

Zenon™ labeling technology utilizes a fluorophore-, biotin- or HRP-labeled Fab fragment directed against the Fc portion of an intact IgG primary antibody in order to form a labeling complex (Figure 1). 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.

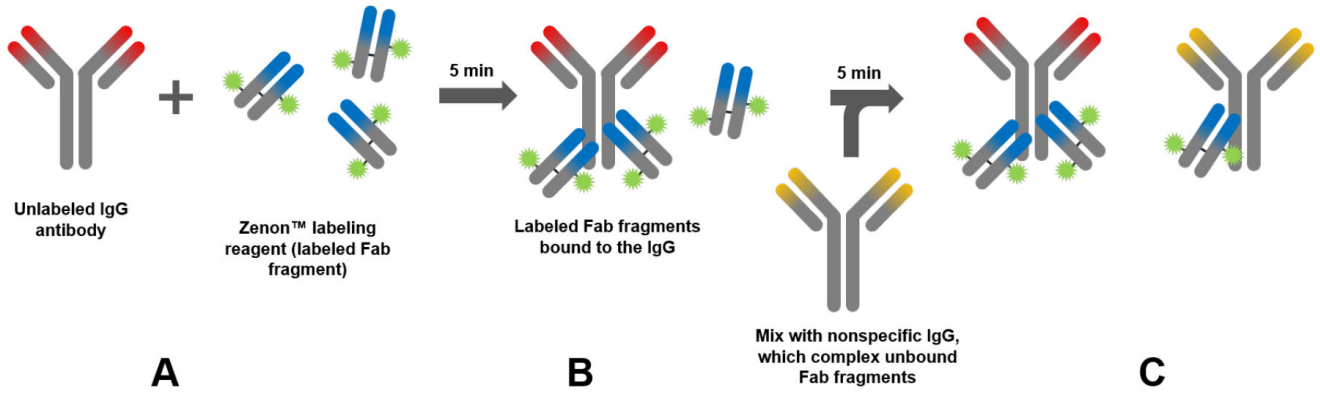

Figure 1 The Zenon™ labeling scheme. (A) An unlabeled IgG is incubated with the Zenon™ labeling reagent, which contains a fluorophore-labeled Fab fragment. (B) The labeled Fab fragment binds to the Fc portion of the IgG antibody. (C) Excess Fab fragment is neutralized by the addition of a nonspecific 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.

Formation of the Fab–antibody complex occurs in less than 5 minutes, and nearly all of the primary antibodies in the mixture are labeled. 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.

Contents and storage

Zenon™ mouse IgG labeling reagents are labeled goat Fab fragments selective for the Fc portion of mouse IgG antibodies of the indicated IgG isotype—IgG_1, IgG_{2a} or IgG_{2b}—depending on the kit.

Note: In the tables below, No. of labelings 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: 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 horseradish peroxidase. When stored as directed, the kits are stable for 6 months after receipt.

Kit	Component	Amount	Concentration	No. labelings	Storage
Zenon™ Mouse IgG Labeling Kits for labeling with Alexa Fluor, Pacific Blue, or Biotin	Zenon™ mouse IgG labeling reagent (Component A)	250 µL	200 µg Fab fragment/mL[1]	50	2°C to 8°C. Protect from light.
	Zenon™ blocking reagent (Component B)	250 µL	5 mg/mL [2]		

[1] Supplied in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide
[2] Supplied in phosphate-buffered saline, pH 7.2, containing 5 mM sodium

Kit	Component	Amount	Concentration	No. labelings	Storage
Zenon™ Mouse IgG Labeling Kits for labeling with R-phycoerythrin (RPE), allophycocyanin (APC), and horseradish peroxidase (HRP)	Zenon™mouse IgG labeling reagent (Component A)	125 µL	200 µg Fab fragment/mL [1]	25	2°C to 8°C. Protect from light. Do not freeze.
	Zenon™ blocking reagent (Component B)	125 µL	5 mg/mL [2]		

[1] RPE and APC conjugates are supplied in 0.1 M sodium phosphate, 0.1 M NaCl, pH 6.8, containing 5 mM sodium azide. HRP conjugates are supplied in 0.1 M sodium phosphate, 0.1 M NaCl, pH 6.8, containing 0.02% thimerosal
[2] Supplied in phosphate-buffered saline, pH 7.2, containing 5 mM sodium

Kit	Component	Amount	Concentration	No. labelings	Storage
Zenon™ Mouse IgG Labeling Kits for labeling with Alexa Fluor™ 647 R-PE tandem constructs	Zenon™ mouse IgG labeling reagent (Component A)	50 µL	200 µg Fab fragment/mL [1]	10	2°C to 8°C. Protect from light. Do not freeze.
	Zenon™blocking reagent (Component B)	50 µL	5 mg/mL [2]		

[1] Supplied in 0.1 M sodium phosphate, 0.1 M NaCl, pH 6.8, containing 5 mM sodium azide.
[2] Supplied in phosphate-buffered saline, pH 7.2, containing 5 mM sodium.

Kit	Component	Amount	Concentration	No. labelings	Storage
Zenon™ Tricolor Mouse IgG Labeling Kits	Zenon™ Alexa Fluor™ 488 Mouse IgG labeling reagent (Component A)	50 µL	200 µg Fab fragment/mL [1]	10	2°C to 8°C. Protect from light.
	Zenon™Alexa Fluor™ 555 Mouse IgG labeling reagent (Component B)				
	Zenon™ Alexa Fluor™ 647 Mouse IgG labeling reagent (Component C)				
	Zenon™blocking reagent (Component D)	150 µL	5 mg/mL [2]		

[1] Supplied in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide.
[2] Supplied in phosphate-buffered saline, pH 7.2, containing 5 mM sodium.

Before you begin

- The Fab:antibody ratio is the important factor when determining the amount of the Zenon™ IgG labeling reagent to use in the labeling protocol. In all Zenon™ IgG Labeling Kits, the Zenon™ labeling reagent is provided at a concentration of 200 µg/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 mixed with 1 µg of IgG antibody produces a Fab:antibody molar ratio of 3:1.
- 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.
- 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™ 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 2 should be determined by using the total IgG mass in the sample to be labeled; thus, 5 µL of the Zenon™ 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.

Zenon™ complex formation protocol

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. 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. 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. 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.
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.)
3. Incubate the mixture for 5 minutes at room temperature.
4. Add 5 µL of the Zenon™ blocking reagent (Component B or Component D if using a Zenon™ Tricolor Kit) to the reaction mixture. The blocking reagent is not isotype specific and is the same in all Zenon™ Mouse IgG Labeling Kits.
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.

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. For imaging experiments, we recommend an additional post-staining fixation step with formaldehyde in order to reduce Fab dissociation. Signal intensity is generally superior with the additional fixation step.

Mounting media. For samples being viewed by microscopy, mount in a suitable antifade mounting medium (e.g., use the ProLong™ Diamond or ProLong™ Glass antifade reagents, Cat. Nos. P36970, P36984).

Spectral data

Table 1 Zenon™ Mouse IgG Labeling Kits.

Label	Ex/Em[1]	Catalog Number		
		Mouse IgG_1	Mouse IgG_{2a}	Mouse IgG_{2b}
Alexa Fluor™ dyes				
Alexa Fluor™ 350	346/442	Z25000	—	—
Alexa Fluor™ 405	402/421	Z25013	—	Z25213
Alexa Fluor™ 488	495/519	Z25002	Z25102	Z25202
Alexa Fluor™ 546	556/573	Z25004	—	—
Alexa Fluor™ 555	555/565	Z25005	Z25105	—
Alexa Fluor™568	578/603	Z25006	—	—
Alexa Fluor™ 594	590/617	Z25007	Z25107	—
Alexa Fluor™ 647	650/668	Z25008	Z25108	Z25208
Alexa Fluor™ 700	696/719	Z25011		
Conventional dyes				
Pacific Blue™	410/455	Z25041	Z25156	
Biotins				
Biotin-XX	NA[2]	Z25052	Z25152	—
R-phycoerythrin, allophycocyanin and tandem constructs				
R-phycoerythrin (R-PE)	496, 546, 565[3]/578	Z25055	Z25155	Z25255
Alexa Fluor™ 647–R-PE	496/668	Z25021	—	—
Allophycocyanin (APC)	650/660	Z25051	Z25151	—
Enzymes				
Horseradish peroxidase	NA	Z25054	—	—

[1] Approximate fluorescence excitation and emission maxima, in nm.
[2] Not applicable.
[3] Multiple absorption peaks.

Table 2 Zenon™ Tricolor Mouse IgG Labeling Kits.

Tricolor Labeling Kit	Labels[1]	Catalog Number		
		Mouse IgG_1	Mouse IgG_{2a}	Mouse IgG_{2b}
Kit #1 for green, orange, and deep red fluorescence imaging	Alexa Fluor™ 488 Alexa Fluor™ 555 Alexa Fluor™ 647	Z25060	—	Z25260

[1] See Table 1, above, for fluorescence excitation and emission maxima.

Life Technologies Corporation | 29851 Willow Creek | Eugene, Oregon 97402 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0025408

Revision	Date	Description
A.0	2 August 2021	New manual