Zenon™ Alexa Fluor™ 647 Goat IgG Labeling Kit

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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 Zenon™ Alexa Fluor™ 647 Goat IgG Labeling Kit provides a fast, versatile and reliable method for producing antibody conjugates, even with very small (sub-microgram) 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 goat-derived antibodies in the same staining protocol.

Zenon™ labeling technology utilizes a fluorophore-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.

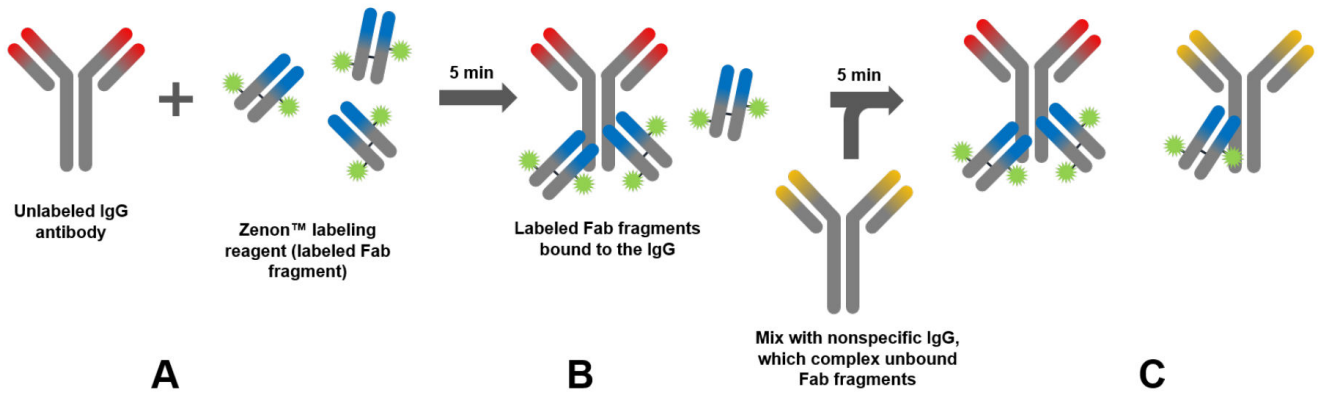

Figure 1 The Zenon™ labeling scheme. An unlabeled IgG is incubated with the Zenon™ labeling reagent, which contains a fluorophore-labeled Fab fragment (A). The labeled Fab fragment binds to the Fc portion of the IgG antibody (B), and excess Fab fragment is neutralized by the addition of a nonspecific IgG (C). The addition of nonspecific IgG prevents cross-labeling of the Fab fragment in experiments where multiple primary antibodies of the same type are present.

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™ Alexa Fluor™ 647 Goat IgG labeling reagents are labeled rabbit Fab fragments specific for the Fc portion of goat IgG antibodies.

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 goat IgG antibody at a Fab:antibody molar ratio of 3:1.

Note: For long-term storage, Zenon™ goat IgG labeling reagents containing low molecular weight fluorophores and the Zenon™ blocking reagent can be divided into single-use aliquots and frozen at ≤–20°C.

Kit	Component	Amount	Concentration	No. labelings	Storage
Zenon™ Alexa Fluor™ 647 Goat IgG Labeling Kit	Zenon™ mouse IgG labeling reagent (Component A)	250 µL	200 µg Fab fragment/mL[1]	50	2°C to 6°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 azide

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

The following protocol is for labeling 1 µg of antibody with a Zenon™ Alexa Fluor™ 647 goat 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™ goat 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 serum may also be directly labeled and do not require purification of the antibody either prior to or after labeling.

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™ goat IgG labeling reagent (Component A) to the antibody solution.
3. Incubate the mixture for 5 minutes at room temperature.
4. Add 5 µL of the Zenon™ blocking reagent (Component B) to the reaction mixture.
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. Goat 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™ Alexa Fluor™ 647 Goat IgG Labeling Kit.

Label	Abs/Em[1]	Catalog Number
Alexa Fluor™ 647	650/668	Z25608

[1] Approximate absorption and emission maxima, in nm.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	2 August 2021	New manual