Zenon™ pHrodo™ iFL IgG Labeling Reagents

Catalog Numbers Z25609, Z25610, Z25611, Z25612, Z25613, and Z25614

Pub. No. MAN0017436 Rev. C

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™ pHrodo™ iFL IgG Labeling Reagents* provide a fast, versatile, and reliable method to evaluate antibody internalization. Zenon™ labeling technology uses a pHrodo™ iFL Red-labeled or pHrodo™ iFL Green-labeled Fab fragment (for example, labeling reagent) directed against the Fc portion of an intact IgG primary antibody to form a labeling complex. Formation of the Fab-antibody complex occurs in less than 5 minutes (Figure 1). Because the pHrodo™ iFL dyes dramatically increase in fluorescence as the pH of their surroundings becomes more acidic, the antibodies coupled with the Zenon™ pHrodo™ iFL IgG Labeling Reagents provide an excellent measure of endocytic process based on the acidification of the labeled antibodies as they are ingested by the cell (Figure 2).

The *Zenon™ pHrodo™ iFL IgG Labeling Reagents* include sufficient material to label one to four 96-well plates, depending on the Zenon™ labeling reagent concentration used.

Procedure overview

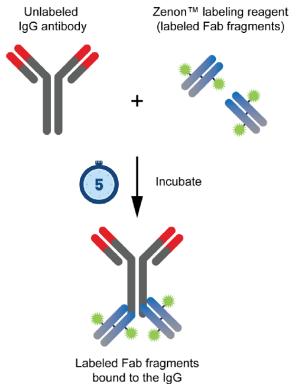

Figure 1 The Zenon™ labeling scheme: An unlabeled IgG is incubated with the Zenon™ pHrodo™ iFL IgG Labeling Reagent, which contains a fluorophore-labeled Fab fragment. The labeled Fab fragment binds to the Fc portion of the IgG antibody.

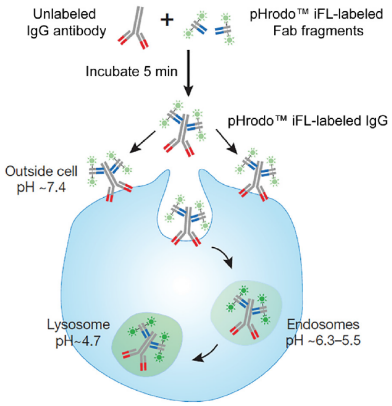

Figure 2 Intact IgG primary antibodies labeled with the *Zenon™ pHrodo™ iFL IgG Labeling Reagents* show a dramatic increase in fluorescence as they are internalized by the cell and the surrounding pH becomes more acidic.

Contents and storage

Zenon™ pHrodo™ iFL IgG Labeling Reagents

Item[1]	Cat. No.	Amount[2]	Storage[3]
Zenon™ pHrodo™ iFL Green Mouse IgG Labeling Reagent	Z25609	250 µL	Store at 2–6°C protected from light. Do not freeze.
Zenon™ pHrodo™ iFL Red Mouse IgG Labeling Reagent	Z25610		
Zenon™ pHrodo™ iFL Green Human IgG Labeling Reagent	Z25611		
Zenon™ pHrodo™ iFL Red Human IgG Labeling Reagent	Z25612		
Zenon™ pHrodo™ iFL Green Human IgG Labeling Reagents	Z25613	1.2 mL	
Zenon™ pHrodo™ iFL Red Human IgG Labeling Reagents	Z25614		

[1] Approximate fluorescence excitation and emission maxima: pHrodo™ iFL Green: 505/530 nm; pHrodo™ iFL Red: 560/585 nm.
[2] 300 µg Fab fragment/mL in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide
[3] When stored as directed, the product is stable for at least 6 months.

Required materials not supplied

- Whole IgG primary antibody
- Suspension cells at 2 x 10^6 cells/mL in cell culture medium or adherent cells in a 96–well plate at 5,000–10,000 cells/well in cell culture medium
- Cell culture medium
- 96-well plates
- Instruments to analyze cells probed with Zenon™ pHrodo™ iFL–labeled IgG (flow cytometer for suspension cells or fluorescence microscope or high-content analysis instrument for adherent cells)

Procedural guidelines

IMPORTANT! pHrodo™ iFL dyes are extremely sensitive to their local environment; therefore, the pH response in the system needs to be determined empirically.

- Zenon™ pHrodo™ iFL IgG Labeling Reagents are specifically designed to selectively target and bind to the Fc portion of human or mouse IgG antibodies. These reagents enable the non-covalent coupling of pHrodo™ iFL Red-labeled or pHrodo™ iFL Green-labeled Fab fragments to unconjugated IgG antibodies within a short period of 5 minutes. This coupling process preserves the antigen binding site of the antibody, ensuring that its functionality remains intact. Additionally, the labeling reagents consistently achieve a labeling density (DOL) of 3–5 Fab molecules per IgG (Figure 1). This level of labeling allows for accurate and reliable detection and analysis of IgG antibodies.
- The labeled Fab fragments (for example, labeling reagents) have been affinity purified during their preparation to ensure their high affinity and selectivity for the Fc portion of the primary antibody. This purification step guarantees the quality and performance of the labeled Fab fragments. Since the 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 before forming the complex. Zenon™ labeling method also demonstrates low cross-reactivity with antibodies from other species, further enhancing its specificity and reliability for antibody labeling applications.
- The formation of the Fab-antibody complex (for example, labeling complex) occurs in less than 5 minutes and nearly all of the primary antibodies in the mixture are labeled. Complexes formed using this technology exhibit fluorescence intensity comparable to that of directly labeled primary antibodies.
- The extent of antibody labeling and the resulting fluorescence intensity of the probe can be adjusted by adjusting the amount of Zenon™ labeling reagent added. This can be achieved by varying the molar ratio of the labeled Fab fragment to primary antibody.
- The protocol provided here, outlines the internalization assays using one 96–well plate of antibodies at a concentration of 40 nM (6 µg/mL) in combination with Zenon™ pHrodo™ iFL IgG labeling reagent at 120 nM (6 µg/mL). This molar ratio is a suggested starting point and represents the minimum ratio for adequate signal in most applications. However, for antibodies targeting highly-expressed or rapidly internalizing antigens, satisfactory signal can be achieved even with lower antibody concentrations.
  - One 96–well plates at 40 nM antibody/120 nM Zenon™ labeling reagent
  - Two 96–well plates at 20 nM antibody/60 nM Zenon™ labeling reagent
  - Four 96–well plates at 10 nM antibody/30 nM Zenon™ labeling reagent

- For larger or smaller quantities of antibody, the amounts of the reagents specified in the protocol can be scaled accordingly. The Zenon™ IgG labeling reaction does not require the removal of bovine serum albumin (BSA) or other stabilizing proteins that can be present in antibody preparations. Antibodies contained within serum can be directly labeled and do not require purification of the antibody either before or after labeling.
- The pHrodo™ iFL Green dye has excitation and emission maxima of approximately 505 nm and 530 nm, respectively. This fluorescence can be detected using standard FITC (fluorescein) or Alexa Fluor™ 488 filters. The pHrodo™ iFL Red dye has excitation and emission maxima of approximately 560 nm and 585 nm. To detect the fluorescence emitted by the pHrodo™ iFL Red dye, standard TRITC (tetramethylrhodamine) or Alexa Fluor™ 555 filters can be used.

Methods

1 Prepare 4X antibody working solution

1. Prepare sufficient volume of 4X working solution of antibody in cell culture medium so that you can use 25 µL for each sample. For example, to fill one 96-well plate, prepare 2.5 mL of working antibody solution.

   Note: 40 nM is a good starting concentration for many antibodies, so a 4X stock will be 160 nM.

2. Aliquot 25 µL of 4X antibody working solution to each well of a 96-well plate.

2 Prepare 4X Zenon™ working solution

1. Prepare 4X working solution of Zenon™ pHrodo™ iFL IgG Labeling Reagent. For example, for one 96-well plate, add 200 µL of Zenon™ pHrodo™ iFL IgG labeling reagent to 2.3 mL of cell culture medium to prepare 2.5 mL of Zenon™ working solution.

2. Aliquot 25 µL of 4X Zenon™ working solution to each well of the 96-well plate from Step 1.2 on page 3. Incubate for 5 minutes at room temperature to allow the labeling complexes to form.

3 Label suspension cells

1. Prepare at least 5 mL of suspension cells at 2×10^6 cells/mL in cell culture medium.

2. Add 50 µL of cells to each well of the 96-well plate containing the antibody and the Zenon™ labeling reagent (from Step 2.2 on page 3).

3. Incubate the cells with the labeling complex for 1–24 hours under standard cell culture conditions. Add other antibodies or cell labels as desired.

4. Analyze cells using flow cytometry.

4 Label adherent cells

1. Prepare a 96-well plate containing 5,000–10,000 cells/well. After the cells adhere, adjust volume so that each well contains 50 µL of culture medium.

2. Add 50 µL of the labeling complex (from Step 2.2 on page 3) to each well of the 96-well plate.

3. Incubate the cells with the labeling complex for 1–24 hours under standard cell culture conditions. Add other antibodies or cell labels as desired.

4. Analyze cells using fluorescence microscopy or high content analysis.

Limited product warranty

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

Revision history: Pub. No. MAN0017436 C

Revision	Date	Description
C	9 October 2024	Added two extra reagents to the product list (Z25613 and Z25614) and minor updates were made throughout the document to improve clarity and consistency of style.
B.0	26 January 2018	Remove definition of labeling from Introduction.
A.0	6 November 2017	New document for *Zenon™ pHrodo™ iFL IgG Labeling Reagents*.