Zenon™ pHrodo™ iFL IgG Labeling Reagents

Catalog Nos. Z25609, Z25610, Z25611, Z25612

Pub. No. MAN0017436 **Rev.** B.0

Product description

The Invitrogen™ Zenon™ pHrodo™ iFL IgG Labeling Reagents provide a fast, versatile, and reliable method to evaluate antibody internalization. Zenon™ labeling technology utilizes a pHrodo™ iFL Red- or pHrodo™ iFL Green-labeled Fab fragment (i.e., 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 activity based on the acidification of the labeled antibodies as they are ingested by the cell (Figure 2).

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

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

Table 1 Contents and storage

Product	Cat. No.	Amount*	Storage**
Zenon™ pHrodo™ iFL Green Mouse IgG Labeling Reagent	Z25609	250 µL	• 2–6°C • DO NOT FREEZE • Protect from light
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		
* 300 µg Fab fragment/mL in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide			
** When stored as directed the product is stable for at least 6 months.			
Approximate fluorescence excitation and emission maxima: pHrodo™ iFL Green: 505/530 nm; pHrodo™ iFL Red: 560/585 nm.			

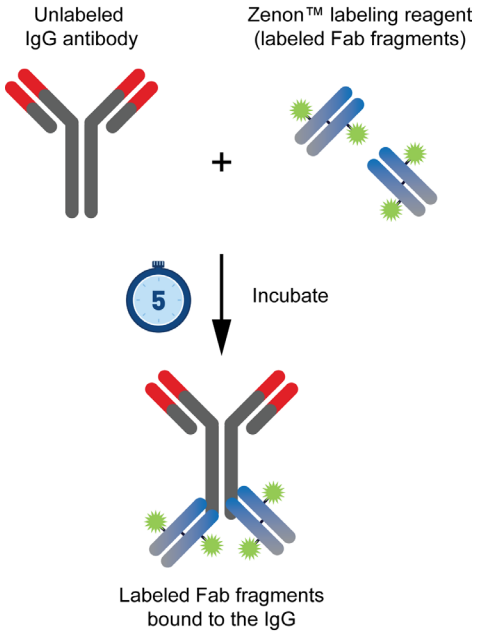

Figure 1. The Zenon™ labeling scheme. An unlabeled IgG is incubated with the Zenon™ pHrodo™ iFL IgG Labeling Reagent, which contains a fluorophore-labeled Fab fragment. The labeled Fab fragment binds to the Fc portion of the IgG antibody.

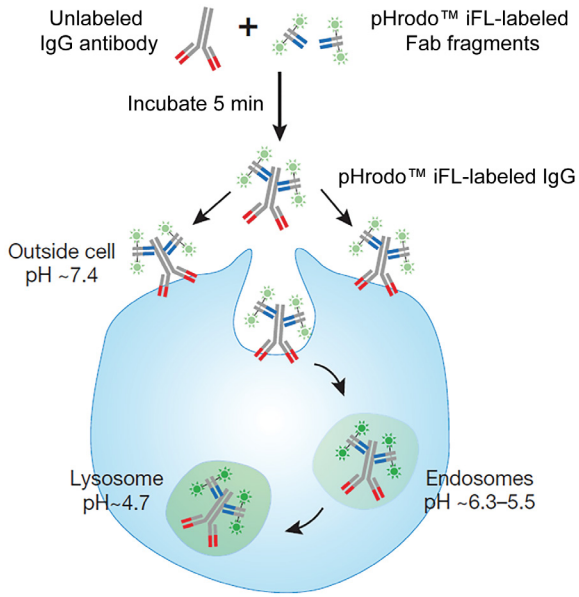

Figure 2. Intact IgG primary antibodies labeled with the Zenon™ pHrodo™ iFL IgG Labeling Reagents show dramatic increases in fluorescence as they are internalized by the cell and the pH of their surroundings become more acidic.

Materials required but not provided

- Whole IgG primary antibody
- Suspension cells at 2×10^6 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

- Zenon™ pHrodo™ iFL IgG Labeling Reagents are goat Fab fragments selective for the Fc portion of human or mouse IgG antibodies. They are used to non-covalently couple the pHrodo™ iFL Red- or pHrodo™ iFL Green-labeled Fab fragments to the unconjugated human or mouse IgG antibodies in 5 minutes, leaving the antigen binding site of the antibody unmodified, while providing a consistent degree of labeling (DOL) of 3 to 5 Fab molecules per IgG (Figure 1).

- The labeled Fab fragments (i.e., labeling reagents) 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. Cross-reactivity is low with antibodies from other species.

- Formation of the Fab–antibody complex (i.e., 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 display fluorescence intensity similar to that of directly labeled primary antibodies.

- The extent of antibody labeling (and thus the fluorescence intensity 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.

- The protocol described here is for performing internalization assays with one 96-well plate of antibodies, each at 40 nM (6 µg/mL) 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. Experiments with antibodies against highly-expressed or rapidly internalizing antigens may obtain satisfactory signal 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 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.

- The pHrodo™ iFL Green dye has excitation and emission maxima of approximately 505 nm and 530 nm, respectively, and can be detected with 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, and can be detected with standard TRITC (tetramethylrhodamine) or Alexa Fluor™ 555 filters.

Methods

Prepare 4X antibody working solution

1.1 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.

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

Prepare 4X Zenon™ working solution

2.1 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.

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

Label suspension cells

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

3.2 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).

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.

3.4 Analyze cells using flow cytometry.

Label adherent cells

4.1 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.

4.2 Add 50 µL of the labeling complex (from Step 2.2) to each well of the 96-well plate.

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

4.4 Analyze cells using fluorescence microscopy or high content analysis.

Ordering information

Cat. No.	Product name	Unit size
Z25609	Zenon™ pHrodo™ iFL Green Mouse IgG Labeling Reagent	250 µL
Z25610	Zenon™ pHrodo™ iFL Red Mouse IgG Labeling Reagent	250 µL
Z25611	Zenon™ pHrodo™ iFL Green Human IgG Labeling Reagent	250 µL
Z25612	Zenon™ pHrodo™ iFL Red Human IgG Labeling Reagent	250 µL

Purchaser notification

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Revision	Date	Description
B.0	26 January 2018	Remove definition of labeling from Introduction.
A.0	06 November 2017	New document

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