pHrodo™ Deep Red Mammalian and Bacterial Cell 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 pHrodo™ Deep Red Mammalian and Bacterial Cell Labeling Kit provides the reagents you need to rapidly label live cells (mammalian or bacterial) with pHrodo™ Deep Red dye to assess phagocytic activity. This kit is optimized for discriminating cells that have been phagocytosed from those that have not, as pHrodo Deep Red only fluoresces upon ingestion in acidic environments. The pHrodo™ dye-based assay provides sensitive detection without the need for quenching reagents, saving time and eliminating the uncertainty of whether signals derive from internalized particles. This kit provides sufficient pHrodo Deep Red dye and wash buffer to perform three live-cell labeling reactions with 1 mL of 1 x 10^6 mammalian cells or 1 mL of 1 x 10^7 bacterial cells.

pHrodo Deep Red is a low-background pH sensor dye that shows no signal in neutral conditions and only fluoresces in acidic environments. This unique property enables rapid assay development and certainty of results investigating endocytic and phagocytic pathways. pHrodo Deep Red enables better discrimination of internalized cargo from outside the cell because it has an approximate pKa of 5 and will not turn on until it enters the phagosome. pHrodo Deep Red dye can be detected using a Cy5 fluorescent filter set and has been verified for use in a variety of applications, including flow cytometry, fluorescent microscopy, and high content screening (HCS).

This user guide describes a general protocol for using the amine-reactive, pHrodo Deep Red Cell Labeling Dye to label either human derived Jurkat cells or *E. coli*. We recommend using this procedure as a starting point. Based on your initial results, you may need to optimize the procedure by varying the incubation time or concentration ratio of fluorophore to cell.

Contents and storage

Contents	Amount	Storage [1]
pHrodo™ Deep Red Cell Labeling Dye (Component A), MW ~1,300 g/mol	3 x 20 µg	• ≤–20°C • Dessicate • Protect from light
Cell Labeling and Wash Buffer (Component B)	50 mL	
Approximate fluorescence excitation and emission maxima of pHrodo Deep Red: 640/655 nm		

[1] The product is stable for at least 6 months when stored as directed.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
For mammalian cell labeling	
Bench top microcentrifuge capable of 400 x g (A centrifuge with a swinging bucket-style rotor is preferable to maximize cell recovery)	MLS
Countess™ cell counter or suitable hemocytometer	AMQAX1000
Trypan Blue	T10282
Cells to be labeled	—
Complete cell culture growth medium	MLS
Sterile 15 mL conical tubes	MLS
For bacterial cell labeling	
Bench top microcentrifuge capable of 500 x g (A centrifuge with a swinging bucket-style rotor is preferable to maximize cell recovery)	MLS
Cultured bacterial cells to be labeled	—
Complete bacterial growth medium	MLS
Sterile 15 mL conical tubes	MLS
1.5 mL Eppendorf centrifuge tubes	MLS

Mammalian cell labeling

This protocol describes the labeling of 1 x 10^6 human derived Jurkat suspension cells with one vial of pHrodo™ Deep Red Cell Labeling Dye.

Before you begin

1. Grow cells as normal in desired media and treatment conditions. Follow regular culturing protocols to keep cells healthy and viable. Avoid overgrowth.
2. Warm Cell Labeling and Wash Buffer (Component B) to 20–25°C.

   The kit will use approximately 15 mL of buffer per labeling reaction.
3. Warm vial(s) of pHrodo™ Deep Red Cell Labeling Dye (Component A) to 20–25°C in advance of adding cells.
4. Cool approximately 5 mL of complete cell culture media per labeling reaction to 4°C in a clean vial.

Harvest and wash mammalian cells for labeling

Work in an aseptic cell culture hood. Start with an appropriate volume of cells at 3 x 10^5 to 4 x 10^5 cells per mL suspended in growth medium.

1. Transfer 5 mL of cell suspension into a sterile 15 mL conical tube, then centrifuge at 400 x *g* for 5 minutes.
2. Carefully aspirate the growth medium from the cells.
3. Screw the cap onto the tube, then tap several times to loosen the pellet.
4. Add 10 mL of pHrodo Deep Red Cell Labeling and Wash Buffer (component B).

   Ensure that the cells are completely dispersed by mixing several times by inversion.
5. Centrifuge at 400 x *g* for 5 minutes, then carefully aspirate the buffer from the cells.
6. Screw the cap onto the tube, then tap several times to loosen the pellet.
7. Add 1 mL of pHrodo Deep Red Cell Labeling and Wash Buffer (component B).

   Ensure that the cells are completely dispersed by mixing several times by inversion.
8. Remove 10 µL of cell suspension and mix with 10 µL of Trypan blue.
9. Transfer 10 µL onto a cell counting slide and determine the cell density and total number of cells in the tube.

   There should be 1 x 10^6 to 2 x 10^6 cells per mL.
10. Remove a volume containing 1 x 10^6 cells and dilute the suspension to a final density of 1 x 10^6 total cells per mL with Labeling and Wash Buffer.

    Note: Only use enough buffer and cell suspension to prepare 1 mL of cells at 1 x 10^6 cells per mL for each labeling reaction.

Label mammalian cells

Each vial of pHrodo Deep Red Cell Labeling dye is sufficient to label 1 x 10^6 cells in 1 mL volume.

1. Transfer the 1 mL cell suspension (1 x 10^6 cells) into the vial of dye, then cap tightly.
2. Mix the reaction gently by inversion until the small pellet of dye at the bottom of the vial is dissolved and no longer visible.
3. Incubate the vial for two hours at 20–25°C, protected from light.

   Note: Some cell types may rapidly internalize surface reacted dye and activate the pH sensor within acidic endosomes, which can contribute to the background signal. For best results, label at ≤25°C on a rocking or rotating platform to prevent an endocytic signal from labeled cells.

   Ideal labeling times may vary between 45 and 180 minutes at temperatures between 4°C and 37°C, depending on the desired application.
4. After the labeling reaction, transfer the cell suspension to a conical tube.

5. Add 1 mL of cold growth medium to the labeling vial and rinse briefly to recover all the cells.

   Note: Transfer the culture medium rinse into the conical tube with cell suspension.

   Note: Serum in the culture medium will help to scavenge unreacted dye, but is not necessary if the cells are cultured in serum free conditions.

6. Centrifuge the cells at 400 x *g* for two minutes, then carefully aspirate the supernatant.

7. Leaving the pellet intact, cap the vial and flick several times to loosen the pellet.

8. Add 2 mL of cold cell culture medium, then resuspend the cells by gently mixing. Do not over mix.

9. Centrifuge the cells at 400 x *g* for two minutes, then carefully aspirate the supernatant.

10. Resuspend the labeled, washed cells in 500 µL of cold cell culture medium.

11. Remove 10 µL of cell suspension and mix with 10 µL of trypan blue.

12. Transfer 10 µL onto a cell counting slide and proceed with cell counting.

13. Resuspend cells to the appropriate final density per mL in complete media for the desired application.

    Cell densities are typically 100,000–500,000 cells per mL for downstream phagocytosis assays.

Bacterial cell labeling

This protocol describes the labeling of 1 x 10^7 *E.coli* cells in one vial of pHrodo™ Deep Red Cell Labeling Dye.

Before you begin

1. Grow *E. coli* or desired bacteria within log phase, keeping track of OD_{600} for cell density.

   An OD_{600} value of 1 is about 5 x 10^8 cells per mL for *E. coli*.

   We recommend maintaining *E. coli* between OD_{600} of 0.5 x 10^8 to 0.8 x 10^8, or 2.5 x 10^8 to 4 x 10^8 cells per mL.

2. Warm pHrodo Deep Red Cell Labeling and Wash buffer to 20–25°C.

   The kit will use approximately 15 mL of buffer per labeling reaction.

3. Warm vial(s) of pHrodo™ Deep Red Cell Labeling Dye (Component A) to 20–25°C.

4. Cool approximately 5 mL of bacterial culture media per labeling reaction to 4°C in a clean vial.

Harvest and wash bacterial cells for labeling

Work with gloves in aseptic conditions to prevent contamination. Start with an appropriate volume of cells at 1 x 10^7 to 1.5 x 10^7 cells per mL suspended in growth medium.

1. Transfer 1 mL of cell suspension into a sterile 15 mL conical tube, then add 4 mL of Cell Labeling and Wash Buffer (Component B).

2. Centrifuge at 500 x *g* for 5 minutes, then carefully aspirate the growth medium from the cells.

3. Screw the cap onto the tube, then tap several times to loosen the pellet.

4. Add 5 mL of Cell Labeling and Wash Buffer (component B).

   Ensure that the cells are completely dispersed by mixing several times by inversion.

5. Centrifuge at 500 x *g* for 5 minutes, then carefully aspirate the buffer from the cells.

6. Screw the cap onto the tube, then tap several times to loosen the pellet.

7. Add 1 mL of Cell Labeling and Wash Buffer (component B).

   Ensure that the cells are completely dispersed by mixing several times by inversion.

Label bacterial cells

Each vial of pHrodo Deep Red Cell Labeling dye is sufficient to label 1 x 10^7 cells in 1 mL volume.

1. Transfer the 1 mL cell suspension (1 x 10^7 cells) into the vial of dye, then cap tightly.

2. Mix the reaction gently by inversion until the small pellet of dye at the bottom of the vial is dissolved and no longer visible.

3. Incubate the vial for two hours at 20–25°C, protected from light.

   Ideal labeling times may vary between 45 and 180 minutes at temperatures between 4°C and 37°C, depending on the desired application.

4. After the labeling reaction, transfer the cell suspension to a conical tube.

5. Add 1 mL of cold bacterial growth medium to the labeling vial and rinse briefly to recover all the cells.

   Note: Transfer the culture medium rinse into the conical tube with cell suspension.

   Note: The culture medium will help to scavenge unreacted dye.

6. Centrifuge the cells at 500 x *g* for two minutes, then carefully aspirate the supernatant.

7. Leaving the pellet intact, cap the vial and flick several times to loosen the pellet.

8. Add 2 mL of cold cell culture medium, then resuspend the cells by gently mixing. Do not over mix.

9. Centrifuge the cells at 500 x *g* for two minutes, then carefully aspirate the supernatant.

10. Resuspend cells to the appropriate final volume in complete media for the desired application.

    Cell volumes are typically 0.5–5 mL per 1 x 10^7 bacterial cells, depending on the downstream phagocytosis assays.

Limited product warranty

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Life Technologies Corporation | 29851 Willow Creek | Eugene, OR 97402
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Revision	Date	Description
A.0	05 November 2020	New document.