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# IncuCyte® pHrodo® Red Cell Labeling Kit for Phagocytosis

Catalog number: 4649

## Contents:

- 1x vial of IncuCyte® pHrodo® Red Cell Labeling Dye
- 1x vial of DMSO used to solubilize the dye (150  $\mu$ L)
- 1x bottle of IncuCyte® pHrodo® Wash Buffer (100 mL)
- 1x bottle of IncuCyte® pHrodo® Labeling Buffer (100 mL)

## Presentation, storage and stability

The IncuCyte® pHrodo® Red Cell Labeling Kit for Phagocytosis contains sufficient reagent for labeling  $5 \times 10^7$  target cells of choice. Component A (IncuCyte® pHrodo® Red Cell Labeling Dye) is supplied as a lyophilized solid which should be stored at  $-20^{\circ}\text{C}$  (stable for at least 6 months). Once solubilized the solution should be used as soon as possible or stored at  $-20^{\circ}\text{C}$  (stable for at least 1 month). The kit contains sufficient DMSO to solubilize the dye, and buffers suitable for washing and labeling cells.

## Background and intended use

IncuCyte pHrodo Red Cell Labeling Dye is a reagent for labeling whole cells with a pH-sensitive fluorophore. These cells are then suitable for use in downstream applications such as Phagocytosis of Cells: [essenbioscience.com/phagocytosis](https://www.essenbioscience.com/phagocytosis)

The unique pHrodo®-based system exploits the acidic environment of the phagosome to quantify phagocytosis. As IncuCyte pHrodo Red labelled cells residing in the neutral extracellular solution (pH 7.4) are engulfed by phagocytes and enter the acidic phagosome (pH 4.5 – 5.5), a substantial increase in fluorescence is observed. In the absence of phagocytes, the fluorescence intensity of the labeled cells remains low. With the IncuCyte® Live-Cell Analysis System integrated analysis software, background fluorescence is minimized. This reagent has been validated for use with a number of cell types. The IncuCyte Live-Cell Analysis System enables real-time evaluation of phagocytic regulation by pharmacological agents as well as genetic and environmental factors.

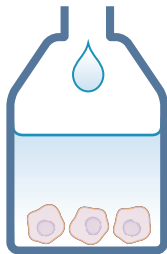
## Recommended use

We recommend that IncuCyte® pHrodo® Red Cell Labeling Dye is prepared at a stock concentration of 1 mg per mL in the sterile DMSO provided. The dye may then be diluted for direct addition to cells suspended in IncuCyte® pHrodo® Labeling Buffer. Note that the dye will also bind to any primary amines present in proteins or cellular debris, therefore we recommend that a) cell lines be washed with IncuCyte® pHrodo® Wash Buffer to remove cell culture media and serum, and b) that any primary cells (such as neutrophils extracted from whole blood) be free from contamination.

Please see the relevant protocol published on our website: [essenbioscience.com/pHrodo-protocols](https://www.essenbioscience.com/pHrodo-protocols)

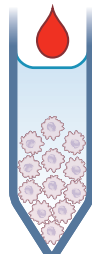
## Quick Guide

### 1 CULTURE TARGET CELLS AND INDUCE APOPTOSIS (OPTIONAL)



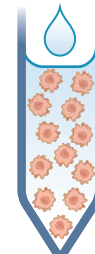
Culture target cells (e.g. Jurkat). (OPTIONAL) Treat with cytotoxic agent (e.g. camptothecin) to induce apoptosis.

### 2 LABEL WITH INCUCYTE® PHRODO RED CELL LABELING KIT



Wash cells with Wash Buffer and add IncuCyte® pHrodo Labeling Dye to apoptotic target cells (e.g. for Jurkats;  $1 \times 10^6$  cells/mL; 250 ng/mL).

### 3 QUENCH AND REMOVE LABEL



Wash cells with Labeling Buffer and resuspend in complete media. Cells are now ready for use in phagocytosis assay.

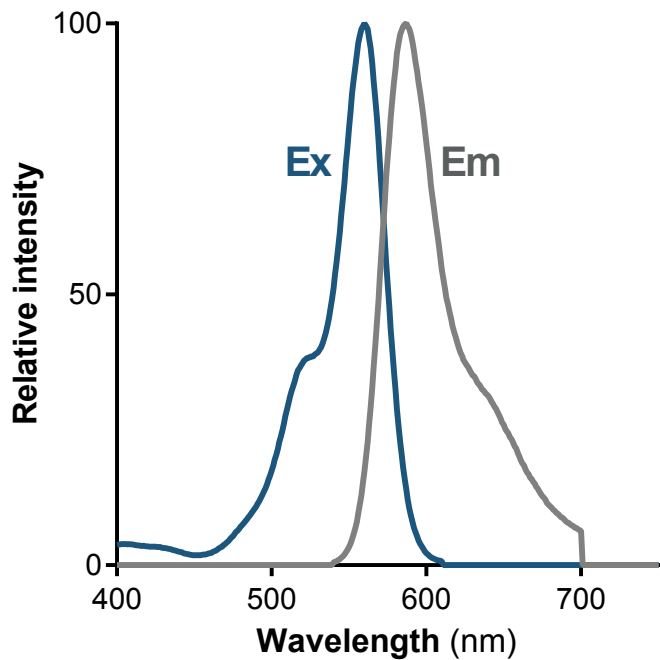
**Figure 1. Overview of labeling protocol.** Target cells (e.g. Jurkats) are treated with a cytotoxic agent if required, to induce apoptosis. Cells are then washed to remove agents, and traces of media and serum. After incubating the cells with labeling reagent for 1 hour, cells are washed with media to quench and remove unreacted dye. Cells are ready for use in subsequent assays, such as phagocytosis of cells (efferocytosis).

## Optimization Protocol: IncuCyte® pHrodo® Red Cell Labeling Kit for Phagocytosis

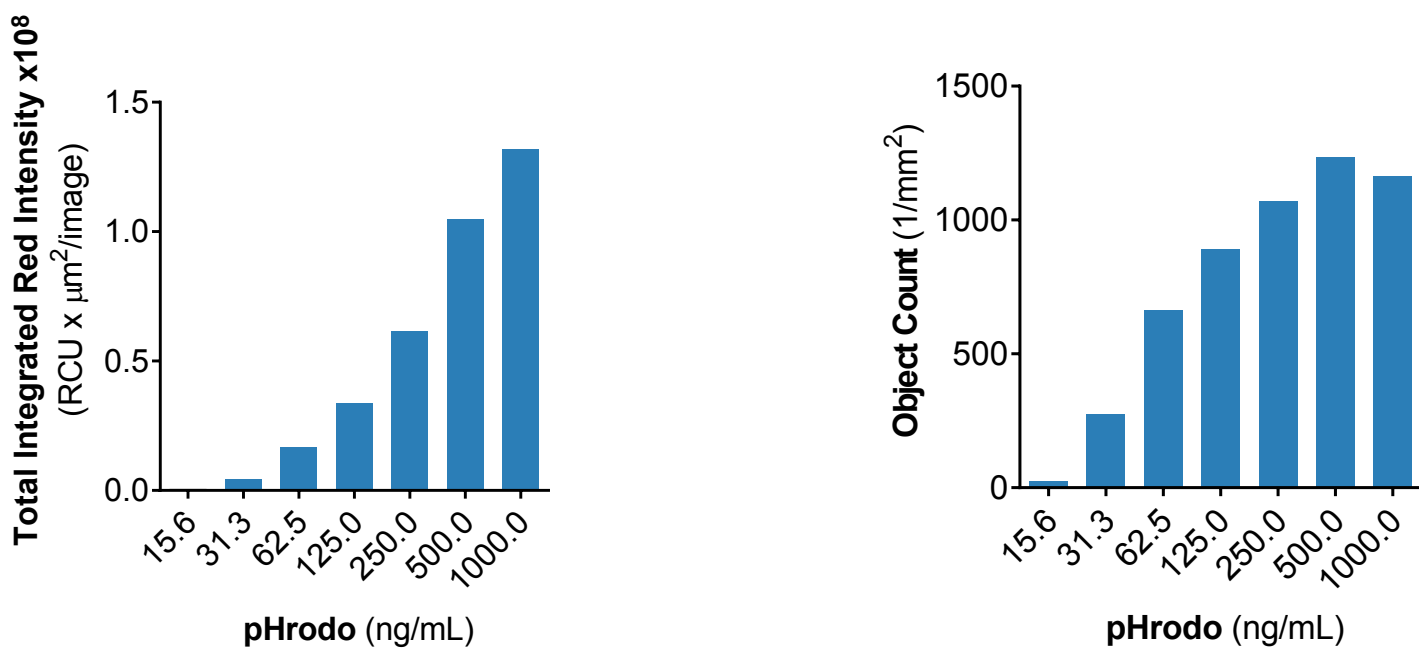
### Protocol Overview:

We have successfully used this method to label a number of target cells including Jurkats, CCRF-CEM, and neutrophils (extracted from blood), however to label other cell types some optimization may be required.

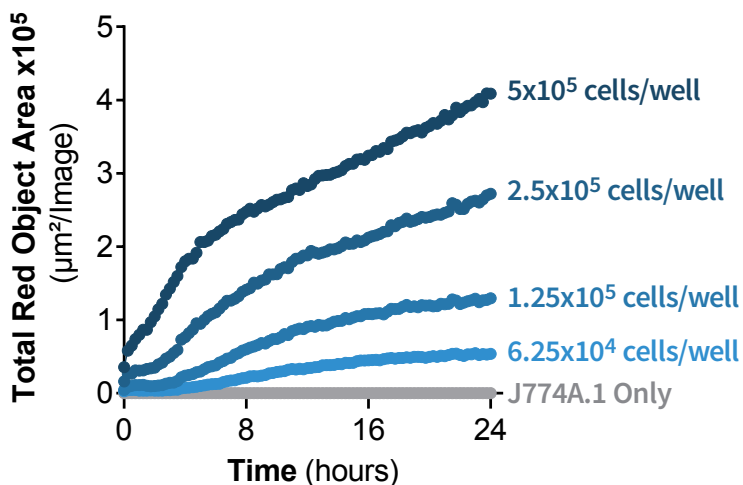
- 1) Suspend cells at a density of  $1 \times 10^6$  cells/ml in IncuCyte pHrodo Labeling Buffer. Separate the suspension into aliquots of 1 mL.
- 2) Solubilize the IncuCyte pHrodo Red Cell Labeling Dye by addition of 100  $\mu$ L of the DMSO provided.
- 3) Perform a serial dilution of the IncuCyte pHrodo Red Cell Labeling Dye in DMSO.
  - a. For cells extracted from blood or tissue, generate a concentration range between 1 mg/mL (stock) and 100  $\mu$ g/mL.
  - b. For cultured cell lines, generate a concentration range between 100  $\mu$ g/mL and 10  $\mu$ g/mL.
- 4) Add 10  $\mu$ L of each concentration of dye to 1 mL cell suspension i.e. a 1:100 dilution, which will provide a final assay concentration range of:
  - a. 10  $\mu$ g/mL to 1  $\mu$ g/mL,
  - b. 1  $\mu$ g/mL to 100 ng/mL.
- 5) Incubate for 1 hour at 37 °C. Harvest cells by centrifugation for 7 minutes, wash with 1 mL complete media (appropriate for cell type) and resuspend in 1 mL complete media.
  - a. A small aliquot (10  $\mu$ L) may be removed and added to buffer of pH 4.0 (100  $\mu$ L). Add 100  $\mu$ L of this solution to a 96-well plate, allow to settle and scan phase and red fluorescence. The fluorescence of the cells at pH 4.0 will be greatly enhanced and will provide an estimate of the labeling efficiency. By counting the number of phase and fluorescent objects, a percentage of labeled cells may be obtained for each concentration of dye.
  - b. The remainder of the labeled target cells may be added to effector cells of choice. Addition of 200  $\mu$ L of  $1 \times 10^6$  target cells/mL (i.e.  $2 \times 10^5$  cells/well) to  $1 \times 10^4$  effector cells/well will generate a target:effector cell ratio of 20:1 which should generate a strong phagocytosis signal.



**Figure 2.** Excitation and emission spectra for the IncuCyte® pHrodo® Red Cell Labeling Dye fluorophore, determined in pH 4.0 buffer.



**Figure 3.** Total integrated red intensity of labeled Jurkats at pH 4.0 (for maximal fluorescence) increases with increasing amounts of IncuCyte pHrodo Red Cell Labeling Dye (A). The number of red objects increases with increasing IncuCyte pHrodo Red Cell Labeling Dye until approximately 250 ng/mL, indicating that a maximal number of cells have been labeled (B).



**Figure 4.** Assay results using neutrophils labelled with 10  $\mu\text{g}/\text{mL}$  IncuCyte pHrodo Red Cell Labeling Dye. Neutrophils were labeled according to the protocol described, then incubated with J774A.1 macrophage cells seeded at  $1 \times 10^4$  cells/well. Cells were monitored in an IncuCyte Live-Cell Analysis System with phase and fluorescence images recorded. Increasing numbers of labeled neutrophils in the presence of phagocytic cells yields greater increase in total red object area as neutrophils are engulfed.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

IncuCyte® pHrodo® Red Cell Labeling Kit (Cat No. 4649) Components	Component	Amount	Storage	Stability
IncuCyte® pHrodo® Red Cell Labeling Dye	A	1 vial	-20 °C	6 Months
Dimethyl sulfoxide (DMSO), anhydrous	B	150 $\mu\text{L}$	18-25 °C	6 Months
IncuCyte® pHrodo® Wash Buffer	C	100 mL	18-25 °C	6 Months
IncuCyte® pHrodo® Labeling Buffer	D	100 mL	18-25 °C	6 Months

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