IncuCyte™ pHrodo® Red Cell Labeling Kit for Phagocytosis

Presentation, storage and stability

The IncuCyte™ pHrodo® Red Cell Labeling Kit for Phagocytosis contains sufficient reagent for labeling 5 x10^7 target cells of choice. Component A (IncuCyte pHrodo Red Cell Labeling Dye) is supplied as a lyophilized solid which should be stored at -20°C (stable for at least 6 months). Once solubilized the solution should be used as soon as possible or stored at -20°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

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® ZOOM integrated analysis software background fluorescence is minimized. This reagent has been validated for use with a number of cell types. The IncuCyte ZOOM live cell imaging platform 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 Red Cell 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 Red Cell 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

Optimization Protocol

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 x10^6 cells/ml in IncuCyte pHrodo Red Cell Labeling Buffer. Separate the suspension into aliquots of 1ml.
2) Solubilize the IncuCyte pHrodo Red Cell Labeling Dye by addition of 100 µl 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 µg/ml.
   b. For cultured cell lines, generate a concentration range between 100 µg/ml and 10 µg/ml.
4) Add 10 µl of each concentration of dye to 1ml cell suspension i.e. a 1:100 dilution, which will provide a final assay concentration range of
   a. 10 µg/ml to 1 µg/ml,
   b. 1 µg/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 µl) may be removed and added to buffer of pH 4.0 (100 µl). Add 100 µl 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 µl of 1 x10^6 target cells/ml (i.e. 2 x10^5 cells/well) to 1 x10^4 effector cells/well will generate a target:effector cell ratio of 20:1 which should generate a strong phagocytosis signal.

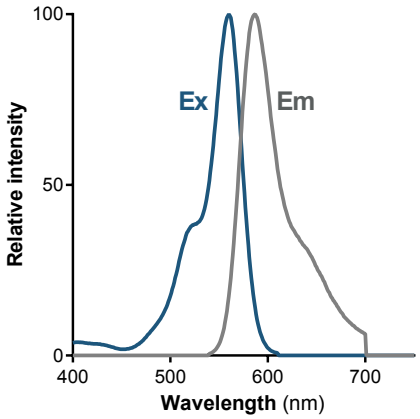

Figure 1. Excitation and emission spectra for the IncuCyte™ pHrodo® green and pHrodo® Red Cell Labeling Dye fluorophore, determined in pH 4.0 buffer.

Safety data sheet (SDS) information

The SDS can be found on our website **essenbioscience.com/phagocytosis**

Quick guide

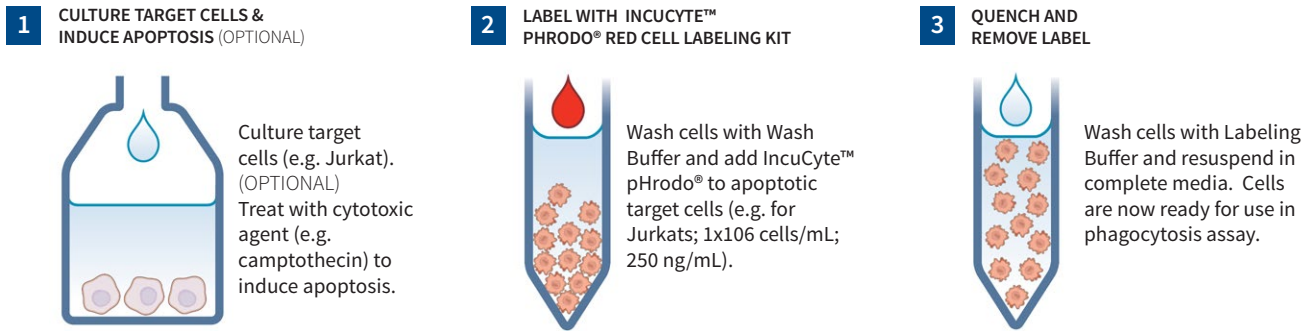

Figure 2. Overview of labeling protocol. Target cells (e.g. Jurkats) are treated with a cytotoxic agent if required, to induce apoptosis. Cells are then washed to remove agents, and traces of media and serum. After incubating the cells with labeling reagent for 1 hour, cells are washed with media to quench and remove unreacted dye. Cells are ready for use in subsequent assays, such as phagocytosis of cells (efferocytosis).

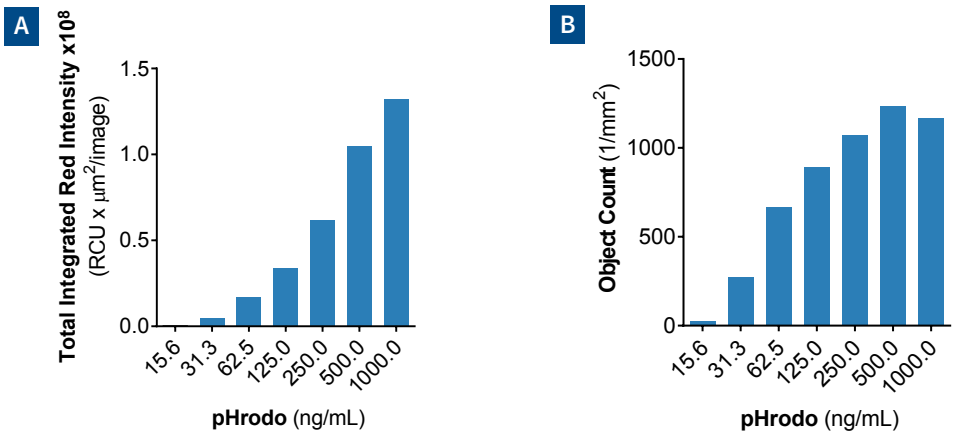

Figure 3. Total integrated red intensity of labeled Jurkats at pH 4.0 (for maximal fluorescence) increases with increasing amounts of IncuCyte™ pHrodo® Red Cell Labeling Dye (A). The number of red objects increases with increasing IncuCyte™ pHrodo® Red Cell Labeling Dye until approximately 250 ng/ml,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

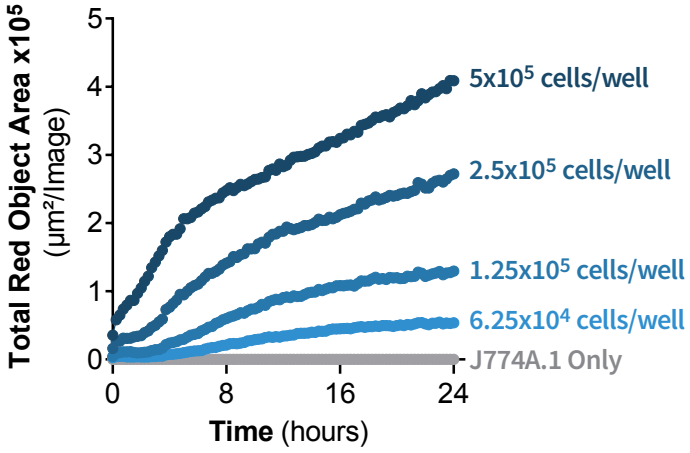

Figure 4. Assay results using neutrophils labelled with 10 µg/ml IncuCyte™ pHrodo® Red Cell Labeling Dye. Neutrophils were labeled according to the protocol described, then incubated with J774A.1 macrophage cells seeded at 1 x10⁴ cells/well. Cells were monitored in an IncuCyte® ZOOM with phase and fluorescence 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 µL	18 - 25 °C	6 months
IncuCyte™ pHrodo® Red Cell Wash Buffer	C	100 ml	18 - 25 °C	6 months
IncuCyte™ pHrodo® Red Cell Labeling Buffer	D	100 ml	18 - 25 °C	6 months

Product label licence

RESEARCH Field

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