molecular probes®
by life technologies™

pHrodo™ Red and Green Dextran for Endocytosis

Catalog nos. P10361, P35368

Table 1 Contents and storage

Material	Cat. no.	Ex/Em Maxima	Amount	Storage*
pHrodo™ Red dextran, 10,000 MW	P10361	560/585 nm	0.5 mg lyophilized powder	• ≤–20°C • Desiccate • Protect from light
pHrodo™ Green dextran, 10,000 MW	P35368	509/533 nm		
* When stored as directed, the product is stable for at least 6 months.				
Number of assays: Sufficient material is supplied for ~25–50 coverslips or 2 microplates, based on the protocol below.				

Introduction

pHrodo™ Red and Green dextran is a superior alternative to other fluorescent dextran conjugates (e.g., BCECF and tetramethylrhodamine [TRITC]) for live-cell imaging of endocytosis, the process whereby the plasma membrane buds to form membrane-bound vesicles, endosomes, which are then trafficked to various destinations within the cell (Figure 1). Endocytosis underlies several fundamental cellular processes including receptor desensitization, nutrient acquisition, and antigen presentation. Furthermore, new roles for endocytosis such as cytokinesis mediation, cell polarization, and cell migration are continuing to be identified.[1]

pHrodo™ Red and Green dextran possesses a pH-sensitive fluorescence emission that increases in intensity with increasing acidity (Figure 2). pHrodo™ dextran is essentially non-fluorescent in the extracellular environment; however, upon internalization, the acidic environment of the endosomes elicits a bright, red- or green-fluorescent signal from this dextran conjugate. The minimal fluorescent signal from the pHrodo™ dextran conjugate at neutral pH prevents the detection of non-internalized and nonspecifically bound conjugates, and eliminates the need for quenching reagents and extra wash steps, thus providing a simple fluorescent assay for endocytotic activity.

pHrodo™ Red and Green dextran can be used to study or monitor endocytosis on a variety of platforms including fluorescence microscopy, flow cytometry, high-throughput screening (HTS), fluorescent microplate reader, and automated imaging and analysis (also known as high-content imaging or HCS) (Figure 3). With appropriate settings, these reagents can also be adapted for bechtop instruments, such as FLoid® Cell Imaging Station, Tali® Image-based Cytometer, and Attune® Acoustic Focusing Cytometer.

Figure 1 Endocytosis/Pinocytosis

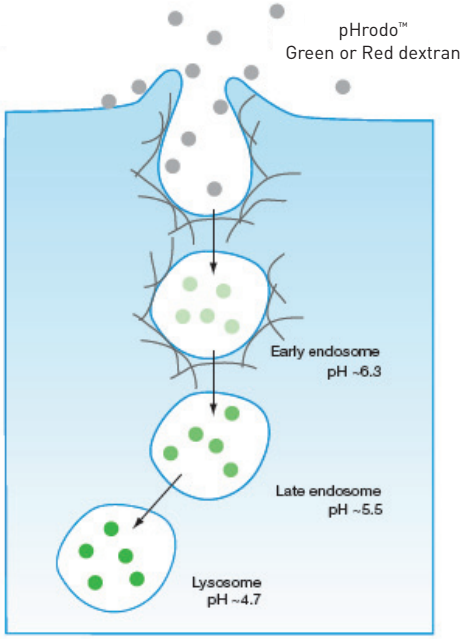
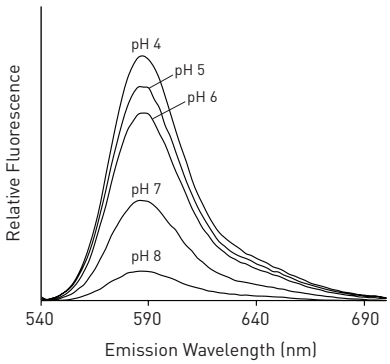
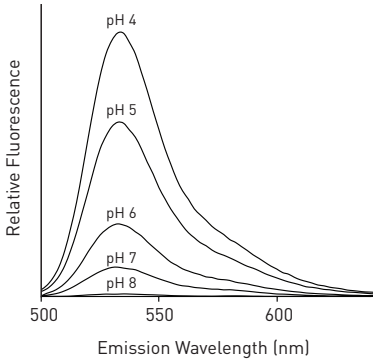


Figure 2 The fluorescence emission spectra of pHrodo™ Red and pHrodo™ Green conjugates



The fluorescence emission spectra of the pHrodo™ Red conjugate



The fluorescence emission spectra of the pHrodo™ Green conjugate

Figure 3 Workflow for pHrodo™ Red and Green dextran conjugates

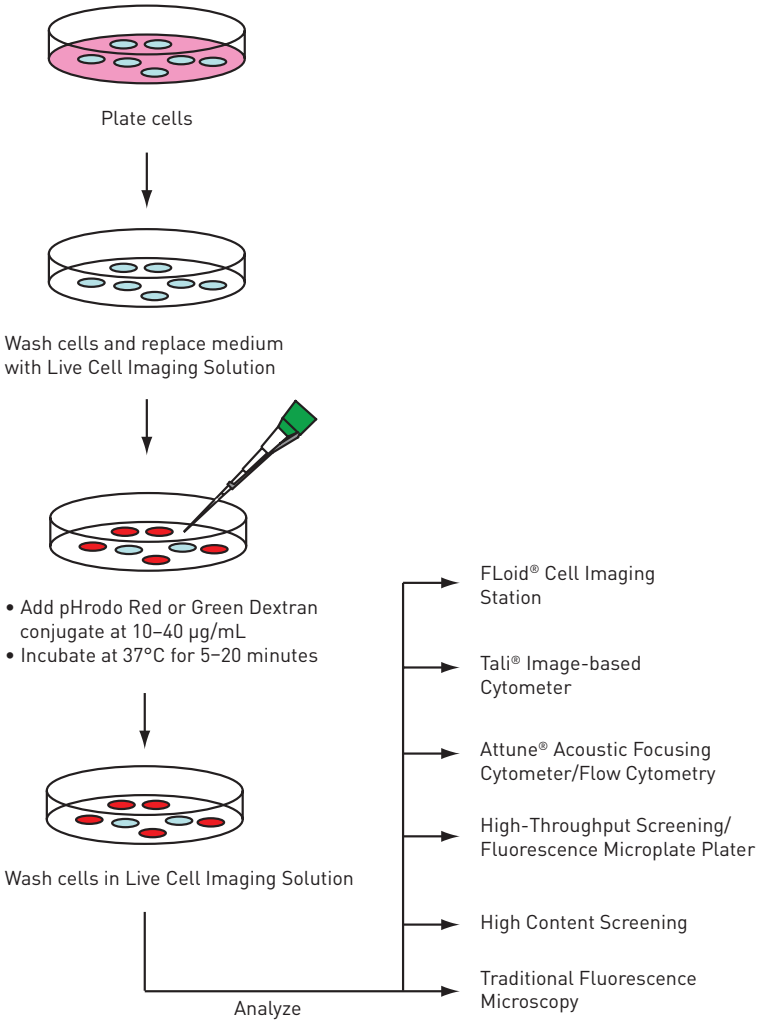
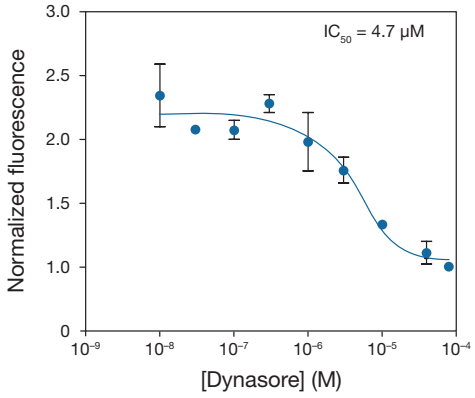


Figure 4 Tracking endocytosis inhibition with pHrodo™ Red dextran conjugate. HeLa cells were plated in 96-well format and treated with dynasore for 3 hours at 37°C prior to the pHrodo™ Red dextran assay. Cells were then incubated with 40 µg/mL pHrodo™ Red dextran for 30 minutes at 37°C, and stained with HCS NuclearMask™ Blue stain for 10 minutes to reveal total cell number and demarcation for image analysis. Images were acquired using the BD Pathway™ 855 High-Content Bioimager (BD Biosciences).



Before Starting

Materials Required but Not Provided

Sterile water or Live Cell Imaging Solution (Cat. no. A14291DJ) to dissolve pHrodo™ dextran for subsequent live-cell imaging experiments.

Preparing Stock Solution

1.1 To prepare a 1 mg/mL pHrodo™ dextran stock solution, dissolve the contents of the entire vial in 500 µL of sterile water or Live Cell Imaging Solution. To minimize bacterial growth, aliquot the stock solution and store the aliquots at ≤–20°C for up to 20 months, protected from light.

Experimental Protocols

Guidelines for Using pHrodo™ Dextran

- Plate cells using standard protocols appropriate for your cell type.
- Before loading the cells with the pHrodo™ conjugate, we recommend changing the medium to Live Cell Imaging Solution for best results. Alternatively, you may use any other appropriate buffer at pH 7.4.
- Determine the optimal concentration of pHrodo™ dextran empirically for a given model; the range suggested in Step 2.2 should be considered a starting guideline.
- Rapid trafficking of pHrodo™ dextran from early endosomes to late endosomes and subsequent fusion with lysosomes can occur. To aid the visualization of pHrodo™ dextran within the endosomes, we recommend increasing the labeling concentration and decreasing the loading time, and imaging immediately.

Labeling Cells with pHrodo™ Dextran

2.1 Wash the cells and replace the growth medium with Live Cell Imaging Solution (Cat. no. A14291DJ). Alternatively, you may use any other appropriate buffer at pH 7.4.

2.2 Add pHrodo™ dextran to the cells at a final concentration of 20–100 µg/mL, and incubate at 37°C for 5–20 minutes.

2.3 Wash the cells with pre-warmed, dye-free medium at pH 7.4 or with Live Cell Imaging Solution.

2.4 Return the cells to dye-free medium at pH 7.4 or Live Cell Imaging Solution, and image the cells using appropriate filters for pHrodo™ dextran.

Note: The fluorescence signal from pHrodo™ dextran is stable for at least one hour after trafficking to lysosomes has occurred. Because lysosomes have a lower pH compared to endosomes, the signal from pHrodo™ dextran within the lysosomes is brighter than the signal from pHrodo™ dextran within the endosomes. The lysosomal pHrodo™ dextran concentration is directly dependent on endocytotic uptake; therefore, the modulation of endocytosis can be inferred from the intensity of pHrodo™ dextran signal from the lysosomes.

Reference

1. Nature Reviews 10, 287 (2009).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
P10361	pHrodo™ Red dextran, 10,000 MW *for endocytosis*	0.5 mg
P35368	pHrodo™ Green dextran, 10,000 MW *for endocytosis*	0.5 mg
Related Products		
A10010	pHrodo™ Red *S. aureus* BioParticles® conjugate for phagocytosis	5 × 2 mg
P35361	pHrodo™ Red *E. coli* BioParticles® conjugate for phagocytosis	5 × 2 mg
P35364	pHrodo™ Red Zymosan A BioParticles® conjugate *for phagocytosis*	5 × 1 mg
P35365	pHrodo™ Green Zymosan A BioParticles® conjugate *for phagocytosis*	5 × 1 mg
P35366	pHrodo™ Green *E. coli* BioParticles® conjugate *for phagocytosis*	5 × 2 mg
P35367	pHrodo™ Green *S. aureus* BioParticles® conjugate *for phagocytosis*	5 × 2 mg
P36600	pHrodo™ Red, succinimidyl ester (pHrodo™ Red, SE)	1 mg
P35362	pHrodo™ Red Avidin *Fluorogenic pH sensor*	1 mg
P35363	pHrodo™ Red Microscale Labeling Kit *Fluorogenic pH sensor* *3 labelings*	1 kit
A14291DJ	Live Cell Imaging Solution	500 mL
R37602	Image-iT® Fixation/Permeabilization Kit	1 kit
R37603	BackDrop™ Background Suppressor *for live cells*	1 kit
R37605	NucBlue™ Live Cell Stain *Hoechst 33342 special formulation*	1 kit
R37606	NucBlue™ Fixed Cell Stain *DAPI special formulation*	1 kit
H10325	HCS NuclearMask™ Blue stain *for 10 × 96-well plates* *2000X concentrate*	65 µL
L3483	Low-density lipoprotein from human plasma, BODIPY® FL complex (BODIPY® FL LDL) *1 mg/mL*	200 µL
L3485	Low-density lipoprotein from human plasma, acetylated, BODIPY® FL conjugate (BODIPY® FL AcLDL) *1 mg/mL*	200 µL
L23380	Low-density lipoprotein from human plasma, acetylated, Alexa Fluor® 488 conjugate (Alexa Fluor® 488 AcLDL) *1 mg/mL*	200 µL
T13342	Transferrin from human serum, Alexa Fluor® 488 conjugate	5 mg
T23366	Transferrin from human serum, Alexa Fluor® 647 conjugate	5 mg
T35357	Transferrin from human serum, Alexa Fluor® 680 conjugate	5 mg

Related Platforms

Attune® Acoustic Focusing Cytometer
(Cat. no. 4469120)

Tali® Image-based Cytometer
(Cat. no. T10796)

FLoid® Cell Imaging Station
(Cat. no. 4471136)

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