Detect pH inside of a live cell

pHrodo® pH sensors for detecting cytosolic and vesicle pH.

Intracellular pH is one of a cell's essential control switches for regulating critical cell functions. Not only does pH affect enzyme activity and other protein functions, but proton gradients are a critical source of energy for driving cell metabolism. Eukaryotic cells contain a variety of defined compartments with different degrees of acidity: intracellular pH is generally between ~6.8 and 7.2 in the cytosol and between ~4.5 and 6.5 in acidic vesicles and organelles. The pH-sensitive pHrodo® dyes are unique pH sensors that undergo a dramatic increase in fluorescence in response to an environmental shift from high to low pH. With a pKa of approximately 6.8, both the pHrodo® Red and pHrodo® Green dyes are useful pH indicators between pH 4 and pH 8, making them ideal for monitoring physiologically relevant pH changes.

The pHrodo® Red and pHrodo® Green dyes are only dimly fluorescent above pH 8; however, as the pH of their surroundings becomes more acidic, their fluorescence intensity increases significantly [1] (Figure 1). As a consequence, pHrodo® Red fluorescence (excitation/emission maxima ~566/590 nm) and pHrodo® Green fluorescence (excitation/emission maxima ~505/520 nm) provide a positive indication of processes such as apoptosis, phagocytic ingestion, and lysosomal sequestration that require acidification, in contrast to the reduction in fluorescence exhibited by fluorescein derivatives. Furthermore, with both red- and green-fluorescent pHrodo® dyes available, you have more flexibility in the design of multiplex cellular assays. Both pHrodo® dyes can be excited with 488 nm light and are compatible with argon-ion lasers commonly installed in flow cytometers, microscopes, and microplate readers.

Detect and calibrate cytosolic pH with pHrodo® AM dyes

To enable cytosolic pH measurements, we have developed cell-permeant forms of the pHrodo® Red and pHrodo® Green dyes (Figure 2). The pHrodo® Red AM and pHrodo® Green AM intracellular pH indicators have been modified with acetoxymethyl (AM) ester groups to produce an uncharged molecule that can permeate cell membranes. Once inside the cell, these lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a pH-sensitive dye that is retained within the intracellular space. Unlike the traditional fluorescein-based pH indicator BCECF AM, pHrodo® Red AM and pHrodo® Green AM exhibit increasing fluorescence as pH decreases and do not photobleach significantly (Figure 3).

To calibrate the fluorescence exhibited by cells loaded with pHrodo® AM dyes (or other intracellular pH indicators), we offer the Intracellular pH Calibration Buffer. This kit contains a selection of calibrated pH solutions (pH 4.5, 5.5, 6.5, and 7.5), as well as the ionophores nigericin and valinomycin, which help equilibrate the pH inside and outside of the cells. After using pHrodo® Red AM or pHrodo® Green AM—or any of the pHrodo® conjugates (see the product list on page 12)—and measuring fluorescence intensity under experimental conditions, the cells are resuspended in one of the calibration buffers to which valinomycin and nigericin have

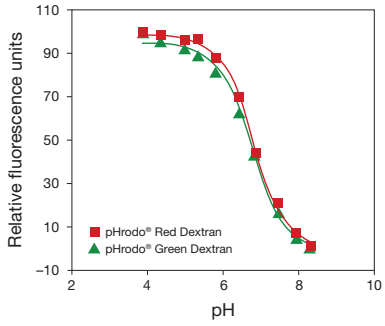

Figure 1. pH response profile of pHrodo® Red and pHrodo® Green dextrans. pHrodo® Red (Cat. No. P10361) and pHrodo® Green (Cat. No. P35368) dextrans were monitored at excitation/emission wavelengths of 550/585 nm and 505/525 nm, respectively, in a fluorescence microplate reader. Citrate, MOPS, and borate buffers were used to span the pH range from 4 to 8.5.

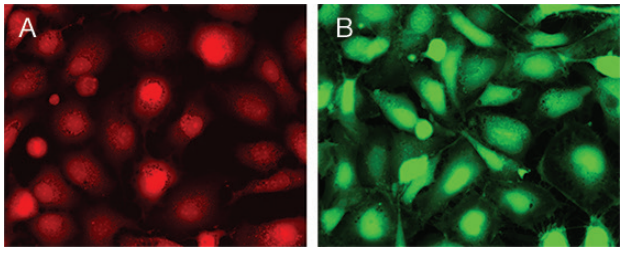

Figure 2. Intracellular pH visualized in live cells. U2OS cells were incubated with **(A)** 5 µM pHrodo® Red AM (Cat. No. P35372) or **(B)** 10 µM pHrodo® Green AM (Cat. No. P35373) intracellular pH indicators using PowerLoad™ Concentrate (provided with the AM esters) for 30 min at room temperature, and then 30 min at 37°C. Both dyes provide an indication of relative pH in different cellular compartments. Acidic compartments and organelles fluoresce more brightly.

been added, and the fluorescence intensity is measured again. This process is repeated with the remaining calibration buffers so that a standard curve can be created and used to pinpoint the intracellular pH associated with the experimental conditions (Figure 4).

Visualizing intracellular pH during apoptosis

A distinctive feature of the early stages of apoptosis is the acidfication of the cytosol, followed by caspase activation and the eventual breakdown of plasma membrane integrity [2, 3]. We have used the intracellular pH indicator pHrodo® Red AM in conjunction with the CellEvent® Caspase-3/7 Green Detection Reagent (a fluorogenic caspase substrate) to monitor a population of HeLa cells undergoing apoptosis. The time course in Figure 5 shows a single cell over the course of 4 hours after treatment with camptothecin. This cell first exhibits increasing pHrodo® Red fluorescence, indicating the acidification of the cytosol, followed by the appearance of the green fluorescence from the caspase-3/7 probe. An adjacent cell was apparently not induced by the camptothecin treatment and remains only dimly fluorescent throughout the time course. →

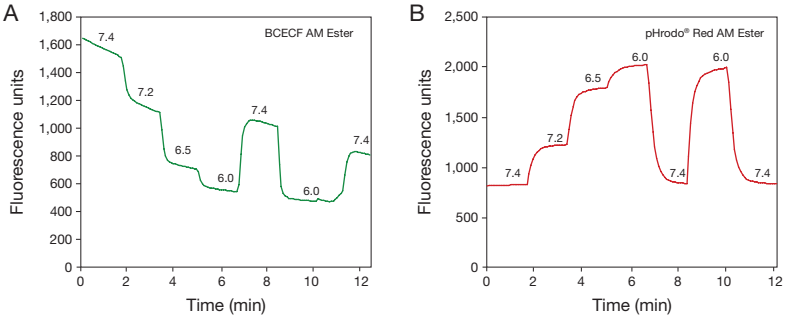

Figure 3. Real-time intracellular pH measurements. NIH/3T3 cells were loaded with **(A)** 5 µM BCECF AM (Cat. No. B1170) or **(B)** 5 µM pHrodo® Red AM (Cat. No. P35372) intracellular pH indicators. Cells were then washed with a series of HEPES-based pH standard buffers containing 10 µM nigericin and 10 µM valinomycin to clamp intracellular pH to the indicated values. BCECF AM fluorescence decreases stepwise as pH is reduced, and decreases continuously due to photobleaching. In contrast, pHrodo® Red AM fluorescence increases as pH is reduced. Furthermore, pHrodo® Red AM does not photobleach significantly after 12 min of imaging.

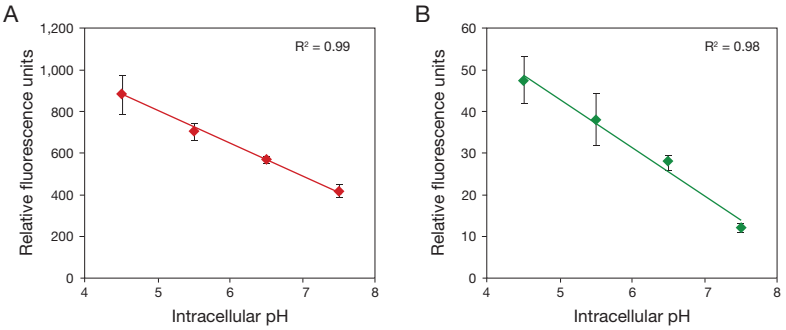

Figure 4. Intracellular pH calibration. U2OS cells in 96-well plates were incubated with **(A)** 5 µM pHrodo® Red (Cat. No. P35372) or **(B)** 10 µM pHrodo® Green AM (Cat. No. P35373) intracellular pH indicators. Standard buffers from the Intracellular pH Calibration Buffer Kit (Cat. No. P35379) were used to clamp the intracellular pH to 4.5, 5.5, 6.5, and 7.5. High-content screening was used to measure mean cellular fluorescence of triplicate samples. A standard curve shows the linear relationship between intracellular pH and relative fluorescence.

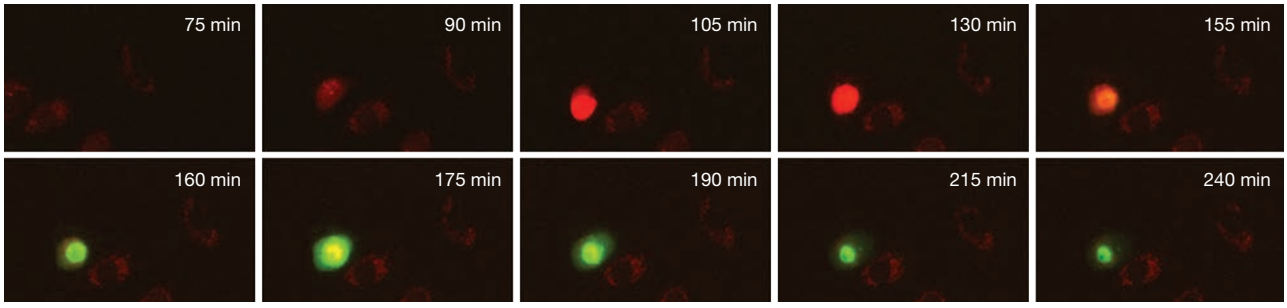

Figure 5. Temporal resolution of intracellular acidification and caspase-3/7 activation in a HeLa cell undergoing apoptosis. HeLa cells were loaded with the intracellular pH indicator pHrodo® Red AM (5 µM, Cat. No. P35372) and the fluorogenic caspase substrate CellEvent® Caspase-3/7 Green Detection Reagent (2 µM, Cat. No. C10423) and then treated with 10 µM camptothecin to induce apoptosis. Cell were imaged continuously over 4 hr on the EVOS® FL Auto Imaging System using a 40x objective and the EVOS® Onstage Incubator. The cell undergoing apoptosis shows increasing pHrodo® Red fluorescence as the intracellular pH drops in the earliest stages of apoptosis, followed by increasing green fluorescence as caspase-3 and -7 are activated. The apparent loss of the pHrodo® Red signal was likely due to compromised membrane integrity and subsequent loss of the dye from the cell.

Detect and monitor pH changes during cellular internalization

The pHrodo® Red and pHrodo® Green dyes are also available as protein and dextran conjugates for studying cellular internalization mechanisms. The ability to study these internalization processes has historically been limited by the lack of tools to directly monitor the ingestion and subsequent acidification of extracellular matter by live cells. We have developed pHrodo® Red and pHrodo® Green dye conjugates that are designed for studying the three primary mechanisms of endocytosis in live cells: 1) pHrodo® conjugates of EGF and transferrin can be used to monitor receptor-mediated endocytosis, 2) pHrodo® dextran conjugates can be used to study fluid-phase endocytosis, and 3) pHrodo® conjugates of bacteria and yeast can be used to track phagocytosis (Figure 6).

Alternatively, you can create your own pH-sensitive bioconjugates using amine- and thiol-reactive derivatives of the pHrodo® Red and pHrodo® Green dyes. Potential applications for pHrodo® bioconjugates include tracking antibody internalization and monitoring virus infection after labeling viral coat proteins.

Explore the role of pH in cell health

pHrodo® pH indicators are ideal live-cell probes for the study of intracellular pH and cellular internalization, and their regulation in both normal development and disease processes. With both red- and green-fluorescent pHrodo® derivatives, you can more easily incorporate pHrodo® dyes into multiplex experiments with other fluorescent live-cell probes, including MitoTracker® mitochondrial probes, CellROX® oxidative stress probes, fluorescent membrane markers, live-cell DNA stains, and CellLight® targeted fluorescent proteins. To learn more about the pHrodo® pH indicators, visit **lifetechnologies.com/phrodobp70**. ■

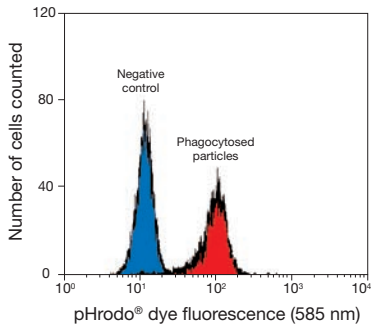

Figure 6. Flow cytometry analysis showing increased fluorescence of granulocytes treated with pHrodo® Red BioParticles® conjugates. A whole blood sample was collected and treated with heparin, and two 100 µL aliquots were prepared. Both aliquots were treated with pHrodo® Red *E. coli* BioParticles® conjugates (Cat. No. P35361) and vortexed. One sample was placed in a 37°C water bath, and the other sample (negative control) was placed in an ice bath. After a 15 min incubation, red blood cells were lysed with an ammonium chloride–based lysis buffer. The samples were centrifuged for 5 min at 500 x *g*, washed once, and resuspended in HBSS. The samples were then analyzed on a BD FACSCalibur™ cytometer using a 488 nm argon laser and 564–606 nm emission filter. The sample incubated at 37°C shows the increased fluorescence of the phagocytosed pHrodo® Red BioParticles® (red), in contrast to the negative control sample, which was kept on ice to inhibit phagocytosis (blue).

References

1. Ogawa M, Kosaka N, Regino CA et al. (2010) *Mol Biosyst* 6:888–893.
2. Ahmad KA, Iskandar KB, Hirpara JL et al. (2004) *Cancer Res* 64:7867–7878.
3. Nilsson C, Johansson U, Johansson AC et al. (2006) *Apoptosis* 11:1149–1159.
4. Suprynowicz FA, Krawczyk E, Hebert JD et al. (2010) *J Virol* 84:10619–10629.

Product	Quantity	Cat. No.
pHrodo® intracellular pH indicators		
pHrodo® Green AM Intracellular pH Indicator	50 µL	P35373
pHrodo® Red AM Intracellular pH Indicator	50 µL	P35372
pHrodo® AM Variety Pack	1 kit	P35380
Intracellular pH Calibration Buffer Kit	1 kit	P35379
pHrodo® BioParticles® conjugates for phagocytosis		
pHrodo® Green E. coli BioParticles® Conjugate, for phagocytosis	5 x 2 mg	P35366
pHrodo® Green E. coli BioParticles® Phagocytosis Kit, for flow cytometry	1 kit	P35381
pHrodo® Green S. aureus BioParticles® Conjugate, for phagocytosis	5 x 2 mg	P35367
pHrodo® Green S. aureus BioParticles® Phagocytosis Kit, for flow cytometry	1 kit	P35382
pHrodo® Green Zymosan A BioParticles® Conjugate, for phagocytosis	5 x 1 mg	P35365
pHrodo® Red Zymosan A BioParticles® Conjugate, for phagocytosis	5 x 1 mg	P35364
pHrodo® Red E. coli BioParticles® Conjugate, for phagocytosis	5 x 2 mg	P35361
pHrodo® Red E. coli BioParticles® Phagocytosis Kit, for flow cytometry	1 kit	A10025
pHrodo® Red S. aureus BioParticles® Conjugate, for phagocytosis	5 x 2 mg	A10010
pHrodo® Red Phagocytosis Particle Labeling Kit, for flow cytometry	1 kit	A10026
pHrodo® dextrans		
pHrodo® Green 10,000 MW Dextran, for endocytosis	0.5 mg	P35368
pHrodo® Red 10,000 MW Dextran, for endocytosis	0.5 mg	P10361
pHrodo® Green Epidermal Growth Factor (EGF) Conjugate	20 µg	P35375
pHrodo® Red Epidermal Growth Factor (EGF) Conjugate	20 µg	P35374
pHrodo® Red Transferrin Conjugate	1 mg	P35376
pHrodo® avidin and reactive pHrodo® dyes		
pHrodo® Red Avidin	1 mg	P35362
pHrodo® Green Maleimide	1 mg	P35370
pHrodo® Green STP Ester	500 µg	P35369
pHrodo® Red Maleimide	1 mg	P35371
pHrodo® Red, Succinimidyl Ester (pHrodo® Red SE)	1 mg	P36600