molecular probes®
by life technologies™

Intracellular pH (pHi) Detection

Table 1 Intracellular pH (pHi) detection products

pHrodo™ Red AM Intracellular pH Indicator (Cat. no. P35372)	**Amount**	**Concentration**	**Storage**
pHrodo™ Red AM Intracellular pH Indicator (Component A)*	1 × 50 µL	5 mM in DMSO	• 2–8°C • Desiccate • Protect from light
PowerLoad™ Concentrate (Component B)	1 × 1 mL	100X	
pHrodo™ Green AM Intracellular pH Indicator (Cat. no. P35373)	**Amount**	**Concentration**	**Storage**
pHrodo™ Green AM Intracellular pH Indicator (Component A)*	1 × 50 µL	10 mM in DMSO	• 2–8°C • Desiccate • Protect from light
PowerLoad™ Concentrate (Component B)	1 × 1 mL	100X	
pHrodo™ AM Variety Pack (Cat. no. P35380)	**Amount**	**Concentration**	**Storage**
pHrodo™ Red AM Intracellular pH Indicator (Component A)*	1 × 10 µL	5 mM in DMSO	• 2–8°C • Desiccate • Protect from light
pHrodo™ Green AM Intracellular pH Indicator (Component B)*	1 × 10 µL	10 mM in DMSO	
PowerLoad™ Concentrate (Component C)	1 × 200 µL	100X	
Intracellular pH Calibration Buffer Kit (Cat. no. P35379)	**Amount**	**Concentration**	**Storage**
Buffer A, pH 4.5 (Component A)	1 × 50 mL	—	• 2–8°C • Dessicate
Buffer B, pH 5.5 (Component B)	1 × 50 mL	—	
Buffer C, pH 6.5 (Component C)	1 × 50 mL	—	
Buffer D, pH 7.5 (Component D)	1 × 50 mL	—	
Valinomycin (Component E)	1 × 5 mg	—	
Nigericin, free acid (Component F)	1 × 5 mg	—	
DMSO, anhydrous (Component G)	1 × 1.3 mL	—	

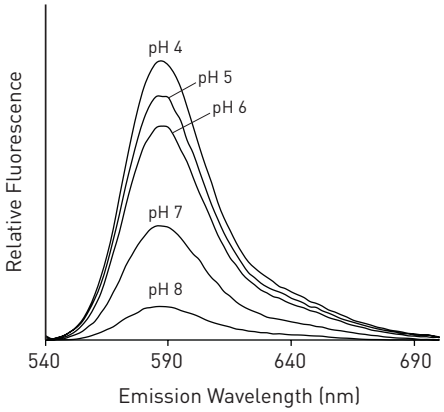
***Approximate Fluorescence Excitation and Emission, in nm:** pHrodo™ Red AM: 560/580, in nm; pHrodo™ Green AM: 509/533, in nm,

Introduction

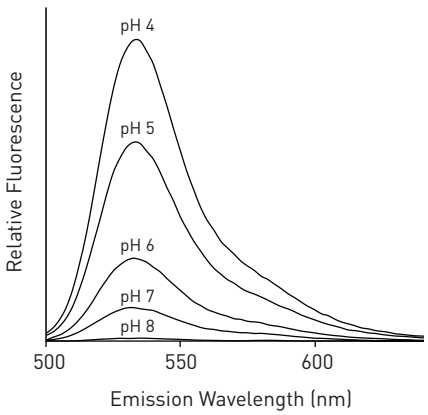
Intracellular pH (pHi) or cytosolic pH is tightly regulated within cells, and many cellular functions are dependent upon proper regulation of intracellular pH.[1,2] Imbalance in intracellular pH can effect many cellular functions, such as ionic homeostasis, reactive oxygen species balance, apoptosis, cell cycle, and cellular mobility.[3–5] pHrodo™ Red AM and pHrodo™ Green AM are novel fluorogenic probes for measuring intracellular pH in live cells. These reagents are weakly fluorescent at neutral pH, but increase their fluorescence with a drop in pH. They have pK_a of ~6.5, and are sensitive to pH change in the range of pH 9–4. Modification of pHrodo™ Red and Green dyes with AM ester groups results in an uncharged molecule that can permeate cell membranes. Once inside the cell, the lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a compound that is retained with the intracellular space. With the help of Intracellular pH buffer kit, this pH change can be quantified.

- The pK_a of ~6.5 is ideally matched to the normal range of cytoplasmic pH (~6.8–7.4).
- Responses to pH across most of cellular function, in the range of pH 9–4.
- With the drop in pH, the fluorescence increases, and the reverse is true if the pH increases. This reversible property makes these dyes ideal for analyzing fluctuating cellular pH levels.
- The fluorescence emission profile of the reagents is pH-dependent (Figure 1, below), allowing the quantification of pH. Intracellular pH Kit can help in this quantification. (see **Intracellular pH Calibration**).
- Excitation and emission properties (Table 1, page 1) make these reagents useful for many platforms, including traditional fluorescence microscopy, high content screening (HCS), flow cytometry, and microplate-based fluorometry or high throughput screening (HTS).
- The acetoxymethyl (AM) ester derivative is membrane permeant, allowing noninvasive bulk loading of cells.
- Once the AM group is cleaved within the cells, the resulting compounds have excellent retention in the cells.
- These compounds are very photo stable, and their narrow spectral properties are ideal for multiplexing with other fluorophores.
- Excellent photostability and retention within the cells eliminate the need to do ratiometric measurements, allowing fluorescence reading at single excitation and emission maxima.

Figure 1 The fluorescence emission spectra of pHrodo™ Red and pHrodo™ Green conjugates

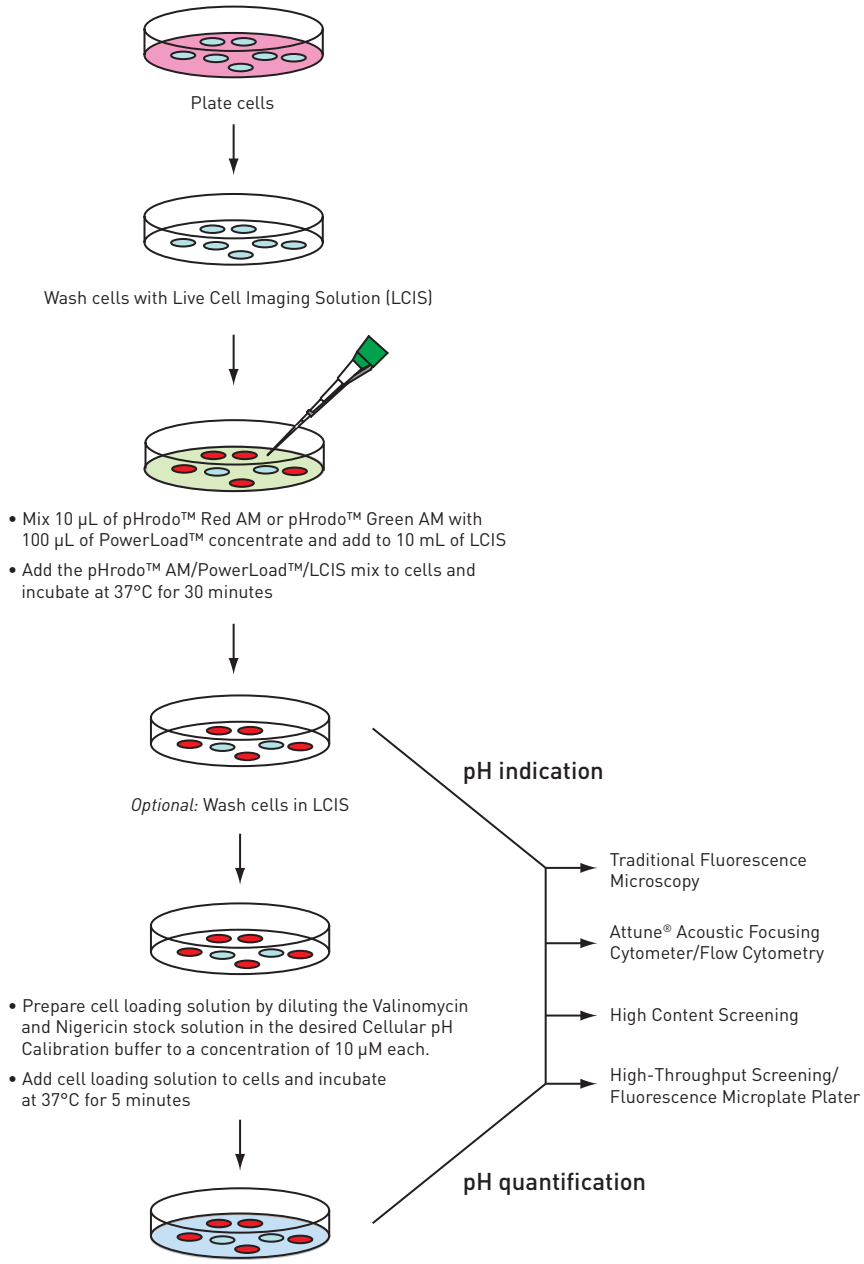


The fluorescence emission spectra of the pHrodo™ Red conjugate



The fluorescence emission spectra of the pHrodo™ Green conjugate

Figure 2 Workflow for intracellular pH (pHi) detection



pH indication: pHrodo™ Red AM and pHrodo™ Green AM

1.1 pHrodo™ Red AM and pHrodo™ Green AM are provided in DMSO as a 1000X solution. Thaw and mix before use.

1.2 Add 10 µL of the pHrodo™ dye to 100 µL of PowerLoad™ concentrate. PowerLoad™ reagent facilitates even cellular loading of AM esters; we recommend its use, but it can be omitted, if needed.

1.3 Dilute the dye solution into 10 mL of Live Cell Imaging Solution (LCIS) (Cat. no. A14291DJ) or other HEPES based pH 7.4 buffer to make a staining solution.

1.4 Remove growth medium from cells and wash once with LCIS.

1.5 Replace LCIS with the pHrodo™ AM staining solution.

1.6 Incubate at 37°C for 30 minutes.

1.7 Wash with LCIS if desired.

1.8 Analyze the cells using the appropriate instrument settings according to Ex/Em maxima in Table 1, page 1.

pH Quantification: Intracellular pH Calibration Buffer Kit

Valinomycin/Nigericin stock solution

2.1 Dissolve Nigericin (Component E) in 345 µL of anhydrous DMSO (Component E) to prepare a 20 mM solution.

2.2 Dissolve Valinomycin (Component F) in 225 µL of anhydrous DMSO (Component E) to prepare a 20 mM solution.

2.3 Combine 100 µL of Nigericin and 100 µL of Valinomycin solutions to prepare a 1000X Valinomycin/Nigericin stock solution (10 mM each). Aliquot and store frozen for up to 1 year.

Cellular labeling

3.1 Prepare Cell Loading Solution by diluting 10 µL of Valinomycin/Nigericin stock solution in 10 mL of the desired Cellular pH Calibration Buffer (Component A, B, C, or D). This cell loading solution contains 10 µM of Valinomycin and 10 µM of Nigericin, in the desired pH calibration buffer.

3.2 Perform the desired cellular experiment with pH-sensitive pHrodo™ AM (Steps 1.1–1.5, above).

3.3 Wash cells 2X with Live Cell Imaging Solution (LCIS) (Cat. no. A14291DJ).

3.4 Replace LCIS with the 10 µM Cell Loading Solution with Valinomycin/Nigericin (from Step 3.1).

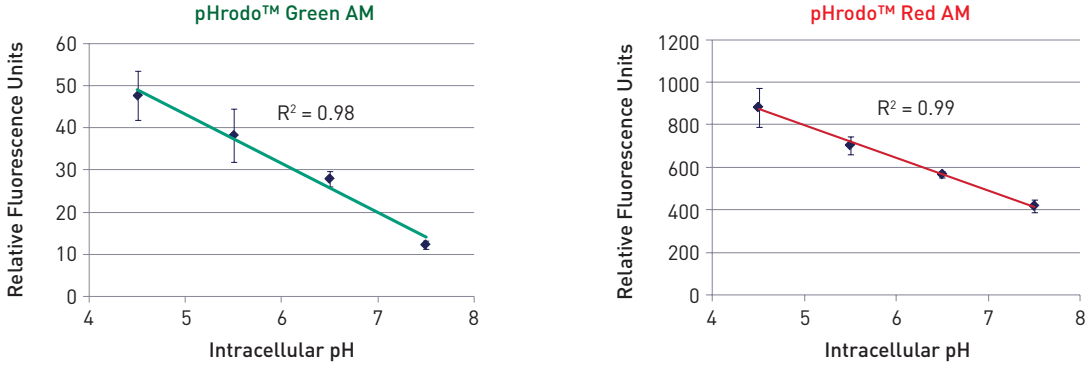
3.5 Incubate at 37°C for at least 5 minutes.

3.6 Analyze the cells using the appropriate Ex/Em maxima.

3.7 Repeat Steps 3.4–3.6 with the three remaining Cellular pH Calibration Buffers (Component A,B,C, or D), if desired. We recommend using at least three pH data points to get a standard curve (Figure 3, below). These data points should yield a plot, and the fluorescence value acquired in Step 3.2 can be plotted in this linear plot to get the cellular pH.

Note: Intracellular pH Calibration Buffer Kit can also be used with other conjugates of pHrodo™ and other intracellular pH indicator dyes such as SNARF® and BCECF.

Figure 3 Standard Curve using pHrodo™ Green AM and pHrodo™ Red AM with Intracellular pH Calibration Buffer Kit. U2OS cells incubated with either 10 µM pHrodo™ Green AM (left) or 5 µM pHrodo™ Red AM (right) for 30 minutes at room temperature, with PowerLoad™ reagent. Intracellular pH Calibration Buffer Kit (Cat. no. P35379) was used to clamp the intracellular pH with extracellular buffer. Four buffers included in the kit, with different pH 4.5, 5.5, 6.5, and 7.5, were used while using a high content screening (HCS) instrument. An average of three data points were plotted in the graph and a linear trendline was fitted to get the pH standard curve. Error bars represent the standard deviation.



References

1. Methods Mol Biol 637, 311 (2010); **2.** Nanotechnology 24, 365 (2013); **3.** Circulation 124, 1806 (2011); **4.** Yonsei Med J 6, 473 (1995); **5.** J Bacteriol 185, 1190 (2003).

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

Cat. no.	Product Name	Unit Size
P35372	pHrodo™ Red AM Intracellular pH indicator	50 µL
P35373	pHrodo™ Green AM Intracellular pH indicator	50 µL
P35379	Intracellualr pH Calibration Buffer Kit	1 kit
P35380	pHrodo™ AM Variety Pack	1 kit
Related Products		
A14291DJ	Live Cell Imaging Solution	500 mL
B1150	2',7'-bis-(2-carboxyethyl) -5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM)	1 mg
B1151	2',7'-bis-(2-carboxyethyl) -5-(and-6)-carboxyfluorescein (BCECF acid) *mixed isomers*	1 mg
B1170	2',7'-bis-(2-carboxyethyl) -5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) *special packaging*	20 × 50 µg
B3051	2',7'-bis-(2-carboxyethyl) -5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) *1 mg/mL solution in anhydrous DMSO*	1 mL
C1270	5-(and-6)-carboxy SNARF®-1	1 mg
C1271	5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester, acetate	1 mg
C1272	5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester, acetate *special packaging*	20 × 50 µg
C6826	5-(and-6)-chloromethyl SNARF®-1, acetate *mixed isomers* *special packaging*	20 × 50 µg
D1878	dextran, BCECF, 10,000 MW, anionic	10 mg
D1880	dextran, BCECF, 70,000 MW, anionic	10 mg
D3303	dextran, SNARF®-1, 10,000 MW, anionic	5 mg
D3304	dextran, SNARF®-1, 70,000 MW, anionic	5 mg
N1495	nigericin, free acid	10 mg
P10361	pHrodo™ Red dextran, 10,000 MW *for endocytosis*	0.5 mg
P35368	pHrodo™ Green dextran, 10,000 MW *for endocytosis*	0.5 mg

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