ab284662– Spexyte™ Intracellular pH Calibration Buffer Kit

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284662

Storage and Stability

On receipt entire assay kit should be stored at +4°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
pH=4.5 Buffer	10 mL	+4°C
pH=5.0 Buffer	10 mL	+4°C
pH= 5.5 Buffer	10 mL	+4°C
pH=6.0 Buffer	10 mL	+4°C
pH=6.5 Buffer	10 mL	+4°C
pH=7.0 Buffer	10 mL	+4°C
pH=7.5 Buffer	10 mL	+4°C
pH=8.0 Buffer	10 mL	+4°C
Nigericin, free acid	1 vial (2mg)	+4°C
DMSO	1 vial (300µL)	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- HH Buffer and pH indicators.
- Fluorescence microscope.
- Black wall/clear bottom plate.
- Fluorescence microplate reader.

Reagent Preparation

- Bring all the kit components to room temperature before starting the experiment.

Preparation of stock solutions:

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at +4°C after preparation. Avoid repeated freeze-thaw cycles.

Nigericin stock solution (10 mM): Add 276 µL of DMSO into the vial of Nigericin (Component I) to make a 10 mM Nigericin stock solution.

Preparation of working solution

Add 1 µL of 10 mM Nigericin stock solution into 1 mL standard pH buffer to make Intracellular pH Calibration Buffer

Sample experimental protocol

Stain cells with BCFL, AM

1. Prepare 5 mM BCFL, AM in DMSO solution.
2. Dilute to 5 µM in HH buffer + 0.02% PF127.
3. Remove growth medium from cells.
4. Add 100 µL BCFL, AM staining solution.
5. Incubate at 37 °C for 30 minutes.
6. Remove BCFL, AM staining solution, and wash once with HH Buffer
7. Empty each well.

Prepare Intracellular pH standard curve

1. Add 100 µL Intracellular pH calibration buffer to cells.

2. Incubate at 37 °C for at 5-10 minutes.

3. Analyse the cells using the appropriate Ex/Em filters.

For example:

BCFL, AM: Ex= 440, 500nm, Em=530nm.

Calculation:

The reading (Ratio) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate pH samples.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)