Spexyte™ Intracellular pH Calibration Buffer Kit

Catalog number: 21235
Unit size: 100 Tests

Component	Storage	Amount
Component A: pH=4.5 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component B: pH=5.0 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component C: pH= 5.5 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component D: pH=6.0 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component E: pH=6.5 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component F: pH=7.0 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component G: pH=7.5 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component H: pH=8.0 Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)
Component I: Nigericin, free acid	Refrigerated (2-8 °C)	1 vial (2 mg)
Component J: DMSO	Refrigerated (2-8 °C)	1 vial (300 µL)

OVERVIEW

Intracellular pH (pHi) plays an important modulating role in many cellular events, including cell volume regulation, cellular metabolism, calcium regulation, receptor-mediated signal transduction, ion transport, endocytosis, and other cellular processes. Intracellular pH is generally 6.8 ~7.4 in the cytosol and 4.5~6.0 in the acidic organelles. Intracellular pH changes have significant physiological effects, e.g., the pH-dependent concentration of intracellular messengers such as Ca2+ and cAMP affects cellular signaling. Several recent reports showed the dysregulated pH is emerging as a hallmark of cancer cells. Spexyte™ Intracellular pH Calibration Buffer Kit provides a range of pH calibration buffers (pH 4.5~ 8.0) with nigericin, which modulate the intracellular pH with the external pH in the presence of 100"150 mM K+. When used in conjunction with pH indicators, such as BCFL, AM or BCECF, AM, Spexyte™ Intracellular pH Calibration Buffer Kit can create a standard curve which is used to determine the intracellular pH.

AT A GLANCE

Protocol Summary

1. Stain cells with pH indicators (for example: BCFL,AM)
2. Wash cells with HH Buffer
3. Prepare Intracellular pH Calibration Buffer
4. Add Intracellular pH Calibration Buffers to cells
5. Incubate at 37 °C for 10 minutes
6. Analyze the cells using the appropriate Ex/Em filter

Important HH Buffer and pH indicators are not provided in this kit.
Bring all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Excitation	Texas Red/FITC filter
Emission	Texas Red/FITC filter
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	440, 500 nm
Emission	530 nm
Cutoff	515 nm
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Nigericin stock solution (10 mM)
Add 276 µL of DMSO (Component J) 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 (Component A to Component H) to make Intracellular pH Calibration Buffer.

SAMPLE EXPERIMENTAL PROTOCOL

Stain cells with BCFL, AM

1. Prepare 5 mM BCFL, AM (Cat#21190) in DMSO solution.
2. Dilute to 5 µM in HH buffer + 0.02% PF127(Cat #20053).
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.

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. Analyze the cells using the appropriate Ex/Em filters. For example: BCFL, AM: Ex= 440, 500nm, Em=530nm.

EXAMPLE DATA ANALYSIS AND FIGURES

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. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

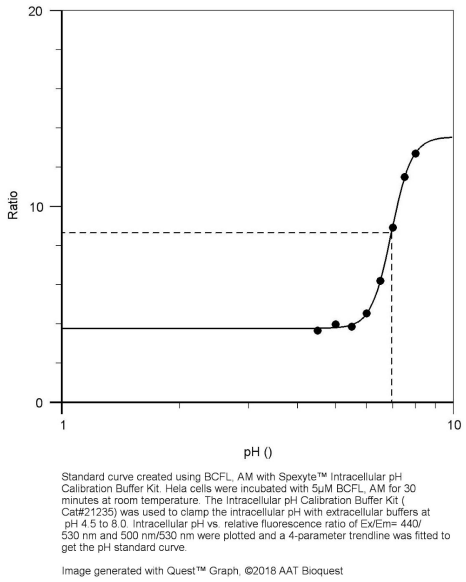

Figure 1. Standard curve created using BCFL, AM with Spexyte™ Intracellular pH Calibration Buffer Kit. Hela cells were incubated with 5μM BCFL, AM for 30 minutes at room temperature. The Intracellular pH Calibration Buffer Kit (Cat#21235) was used to clamp the intracellular pH with extracellular buffers at pH 4.5 to 8.0. Intracellular pH vs. relative fluorescence ratio of Ex/Em= 440/ 530 nm and 500 nm/530 nm were plotted and a 4-parameter trendline was fitted to get the pH standard curve.

DISCLAIMER

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