# Spexyte™ Intracellular pH Calibration Buffer Kit

Catalog number: 21235
Unit size: 100 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: pH=4.5 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component B: pH=5.0 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component C: pH= 5.5 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component D: pH=6.0 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component E: pH=6.5 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component F: pH=7.0 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component G: pH=7.5 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component H: pH=8.0 Buffer | Refrigerate (2-4 °C) | 10 mL |
| Component I: Nigericin, free acid | Refrigerate (2-4 °C), Dessicated | 2 mg |
| Component J: DMSO | Freeze (<-15 °C) | 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

| | |
|---|---|
| Instrument: | Fluorescence microscope |
| Instrument specification(s): | Texas Red/FITC filter |
| Recommended plate: | Solid black/clear bottom |
| Instrument: | Flow cytometer |
| Instrument: | Fluorescence microplate reader |
| Excitation: | N/A |
| Emission: | N/A |
| Recommended plate: | Solid black/clear bottom |

## 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.*

*1. 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 10mM Nigericin stock solution into 1 mL standard pH buffer (Component A to Component H) to make Intracellular pH Calibration Buffer.

## PREPARATION OF CELL SAMPLES

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

## SAMPLE EXPERIMENTAL PROTOCOL

This is a sample experimental protocol to calibrate Intracellular pH with BCFL, AM pH indicator.

**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.
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.

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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

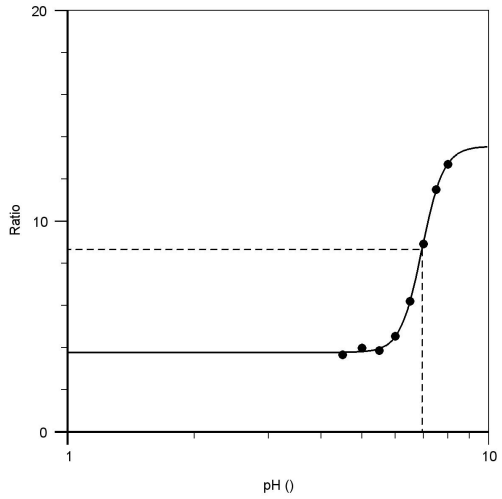


**Figure 1.** Standard curve created using BCFL, AM with Spexyte™ Intracellular pH Calibration Buffer Kit. Hela cells were incubated with 5µM BCFL, AM for 30 minutes at room temperature. The Intracellular pH Calibration Buffer Kit ( Cat#21235) was used to clamp the intracellular pH with extracellular 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