Protonex™ Red 670 AM *Cell-Permeable*

Catalog number: 21182
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21182)
Protonex™ Red 670 AM	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Protonex™ Red 670 AM is the cell-permeable version of Protonex™ Red 670. Protonex™ Red 670 dye works by changing its fluorescence intensity depending on the pH of the environment. Protonex™ Red 670 is minimally fluorescent at a basic pH and maximally fluorescent at an acidic pH. When Protonex™ Red 670 is bound to an acidic intracellular target, it becomes highly fluorescent and emits red light when excited by a red laser such as a 632 nm He-Ne or 647 nm red laser. By measuring the fluorescence intensity of Protonex™ Red 670, one can label or monitor the acidic intracellular targets in live cells.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 Filter Set
Excitation	Cy5 Filter Set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	665 nm
Emission	680 nm
Excitation	640 nm
Recommended plate	Black wall/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

Protonex™ Red 670 AM Stock Solution

1. Prepare a 10 to 20 mM stock solution of Protonex™ Red 670 AM in high-quality anhydrous DMSO.

   Note: An unused Protonex™ Red 670 AM stock solution should be divided into single-use aliquots and stored at ≤ -20 ° C. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Protonex™ Red 670 AM Working Solution

1. On the day of the experiment, either dissolve Protonex™ Red 670 AM dye in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a Protonex™ Red 670 AM dye working solution of 5 to 20 µM in a buffer of your choice (e.g., Hanks and Hepes buffer).

   Note: The nonionic detergent Pluronic® F-127 can be used to increase the aqueous solubility of AM esters. The final Pluronic® F-127 concentration should be approximately 0.02% in the staining buffer. A variety of Pluronic® F-127 products can be purchased from AAT Bioquest (here). Avoid long-term storage of AM esters in the presence of Pluronic® F-127.

   Note: If your cells contain organic anion-transporters, probenecid (1-4 mM) may be added to the dye working solution (final in well concentration will be 0.5-2 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest (here).

SAMPLE EXPERIMENTAL PROTOCOL

Important

The following is a recommended protocol for loading Protonex™ Red 670 AM dye into live mammalian cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare viable cells as desired (e.g., 100 µL/well/96-well plate or 25 µL/well/384-well plate).

2. On the next day, add the Protonex™ Red 670 AM dye working solution into the cell plate in equal volumes, such as 100 µL/well/96-well plate or 25 µL/well/384-well plate.

3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.

4. Replace the dye working solution with HHBS or buffer of your choice to remove any excess dye.

5. Prepare the compound plates using HHBS or a buffer of your choice.

6. Perform the pH assay as desired while simultaneously monitoring fluorescence. This can be done with either a fluorescence microscope that has a Cy5 filter set or a fluorescence plate reader at Ex/Em = 640/680 nm (cutoff 665 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

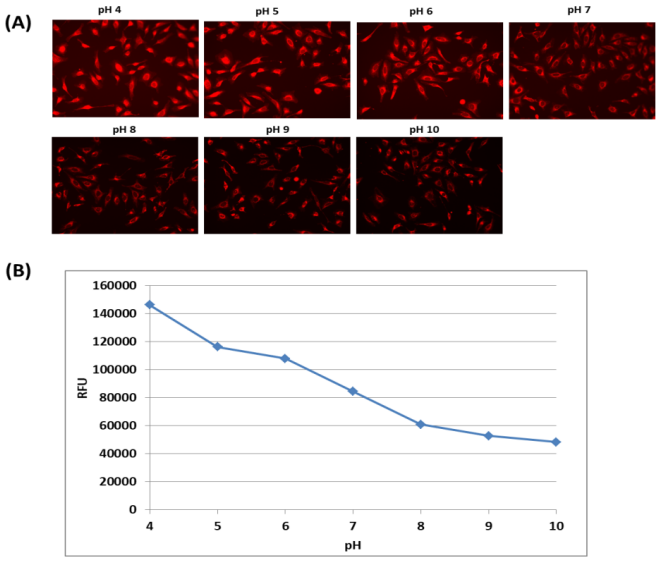

Figure 1. Response of HeLa cells labeled with Protonex™ Red 670 AM. HeLa cells were incubated with 5 µM of Protonex™ Red 670 AM for 30 minutes at 37°C. Incubation of Protonex™ Red 670 AM solution with HeLa cells showed a homogenous uptake of Protonex™ Red 670 AM and stained cell cytosol. The Spexyte™ Intracellular pH Calibration Buffer Kit (Cat No. 21235) was used to clamp the intracellular pH with extracellular buffers at pH 4 to 10. (A) Images were acquired using a fluorescence microscope with a Cy5 filter set, and (B) fluorescence was measured using a ClarioStar fluorescence microplate reader (Ex/Em = 640/680 nm, cutoff = 665 nm).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.