

**Protonex™ Lyso-Red 670**

Catalog number: 21241

Unit size: 1 mg

Component	Storage	Amount (Cat No. 21241)
Protonex™ Lyso-Red 670	Freeze (< -15 °C), Minimize light exposure	1 mg

**OVERVIEW**

Lysosomes are membrane-bound organelles containing acid hydrolases that break down biomolecules in an acidic environment (pH 4.5-4.8). They play critical roles in cellular processes by eliminating waste and debris, facilitating autophagy for organelle recycling, and mediating digestion through endocytosis and phagocytosis. Lysosomes aid cellular metabolism by converting complex molecules into usable forms and contribute to cell signaling, influencing growth, differentiation, and responses to signals. Dysfunctional lysosomes are associated with various diseases called lysosomal storage disorders, where the accumulation of undigested substances within lysosomes leads to cellular damage and systemic health issues. Notably, lysosomal dysfunction has been implicated in neurodegenerative diseases, cancer, and other disorders. Protonex™ Lyso-Red 670 is a cell-permeable, far-red fluorescent probe that selectively stains and visualizes acidic organelles within live cells, notably lysosomes. It remains quiescent or weakly fluorescent under alkaline pH conditions, exhibiting significant fluorescence enhancement as acidity increases. This property enables researchers to trace lysosomal dynamics (e.g., trafficking, fusion, fission, autophagy) in real-time via fluorescence microscopy techniques. Protonex™ Lyso-Red 670 has an excitation and emission maximum of 640 and 680 nm, respectively, and can be efficiently excited using a Cy5 filter.

**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™ Lyso-Red 670 Stock Solution**

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

**Note:** An unused Protonex™ Lyso-Red 670 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™ Lyso-Red 670 Working Solution**

1. On the day of the experiment, either dissolve Protonex™ Lyso-Red 670 in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a Protonex™ Lyso-Red 670 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. In the staining buffer, the final Pluronic® F-127 concentration should be approximately 0.02%. 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.

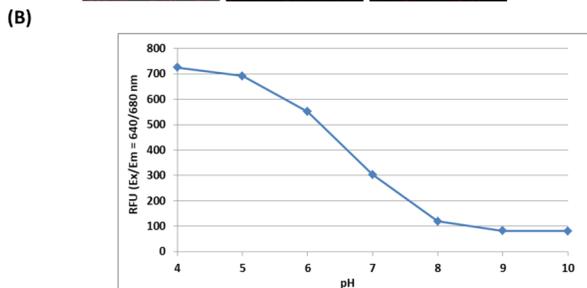
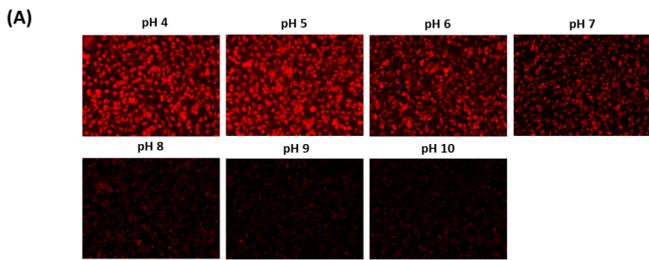
**Note:** If your cells contain organic anion-transporters, probenecid (1-4 mM) may be added to the dye working solution (final in well concentration will be 0.5-2 mM) to reduce leakage of the de-esterified indicators. A variety of ReditUse™ probenecid products, including water-soluble, sodium salt,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

**SAMPLE EXPERIMENTAL PROTOCOL**
**Important**

The following is a recommended protocol for loading Protonex™ Lyso-Red 670 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 {eg. 100 μL/well (96-well plate) or 25 μL/well (384-well plate)}.
2. On the next day, add equal volumes, such as 100 μL/well (96-well plate) or 25 μL/well (384-well plate) of the Protonex™ Lyso-Red 670 working solution, into the cell 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 probes.
5. Prepare the compound plates using HHBS or a buffer of your choice.
6. Run the pH assay as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with 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™ Lyso-Red 670. HeLa cells were incubated with 5  $\mu$ M of Protonex™ Lyso-Red 670 for 30 minutes at 37°C. Incubation of Protonex™ Lyso-Red 670 solution with HeLa cells showed a homogenous uptake of Protonex™ Lyso-Red 670 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. Images were acquired using a fluorescence microscope with a Cy5 filter set (A), and fluorescence was measured using a fluorescence microplate reader (FlexStation 3) at Ex/Em = 640/680 nm, cutoff = 665 nm (B).

**DISCLAIMER**

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