Protonex™ Red 600-Dextran Conjugate *10,000 MW for Endocytosis*

Catalog number: 21236
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21236)
Protonex™ Red 600-Dextran Conjugate *10,000 MW for Endocytosis*	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Protonex™ Red 600-Dextran Conjugate *10,000 MW for Endocytosis* is a pH-sensitive red fluorescent dextran dye designed for real-time, wash-free imaging of endocytosis, phagocytosis, and lysosomal acidification in live-cell assays. This next-generation pH-responsive probe remains nonfluorescent at neutral pH but yields an intense red signal upon internalization into acidic compartments (e.g., endosomes and lysosomes), eliminating the need for secondary quencher dyes or disruptive wash steps. Its Texas Red®-compatible spectral profile ensures seamless integration with standard microscopy filters, flow cytometry systems, and automated plate readers—perfect for multiplexing with green (FITC, GFP) or far-red fluorophores in multi-parameter assays.

AT A GLANCE

Protocol Summary

1. Prepare cells in a growth medium
2. Replace the medium with Protonex™ Red 600-Dextran loading solution (100 µL/well for 96-well plate)
3. Incubate at 37ºC for 5–20 minutes
4. Read Fluorescence at Ex/Em = 576/597 nm

KEY PARAMETERS

Fluorescence microscope

Emission	576 nm (Texas Red®-compatible)
Excitation	597 nm (Texas Red®-compatible)
Recommended plate	Black wall/clear bottom

CELL PREPARATION

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

SAMPLE EXPERIMENTAL PROTOCOL

Assay Protocol for Endocytosis

The following protocol is recommended for standard cell loading. It serves as a general guideline and may be adapted to suit specific experimental requirements.

1. Prepare cells as desired:

1. For example, plate adherent cells overnight in growth medium at 40,000 to 80,000 cells/well/100µL for a 96-well plate or 10,000 to 20,000 cells/well/25µL for 384-well plates.

   Note: Optimal cell density may vary by cell line and should be determined experimentally.

2. Prepare Protonex™ Red 600-Dextran loading solution:

1. Prepare a 1mg/mL stock solution of Protonex™ Red 600-Dextran in 1 mL of sterile water or Hanks and 20 mM Hepes buffer (HHBS). The stock solution should be used promptly. Any unused solution need to be aliquoted and refrozen at < -20 oC.

   Note: Avoid repeated freeze-thaw cycles, and protect from light.
2. Prepare a 20-100ug/mL Protonex™ Red 600-Dextran loading solution in HHBS.

3. Run Endocytosis Assay:

1. Remove the medium, and add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Protonex™ Red 600-Dextran loading solution into the cell plate (from Step 2.2).

   Note: It is important to replace the growth medium with HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384-well plate before dye-loading) if your compounds interfere with the serum.

   Note: Rapid trafficking of Protonex™ Red 600-Dextran from early endosomes to late endosomes and subsequent fusion with lysosomes can occur. To aid the visualization of Protonex™ Green dextran within the endosomes, we recommend increasing the labeling concentration and decreasing the loading time, and imaging immediately.
2. Incubate the dye-loading plate at cell incubator for 5 to 20 minutes.
3. Wash and replace the dye-loading solution with HHBS or growth medium.
4. Run the endocytosis assay by monitoring the fluorescence at Ex/Em = 576/597 nm.

Notes:

- Protonex™ Red 600-Dextran becomes bright red under acidic conditions (e.g., in lysosomes), enabling monitoring of endocytosis and lysosomal acidification.
- The fluorescence signal is relatively stable for at least one hour after trafficking to lysosomes.
- Because lysosomes have a lower pH than endosomes, lysosomal staining is typically brighter. Modulation of endocytosis or lysosomal function can be inferred by changes in fluorescence intensity.

EXAMPLE DATA ANALYSIS AND FIGURES

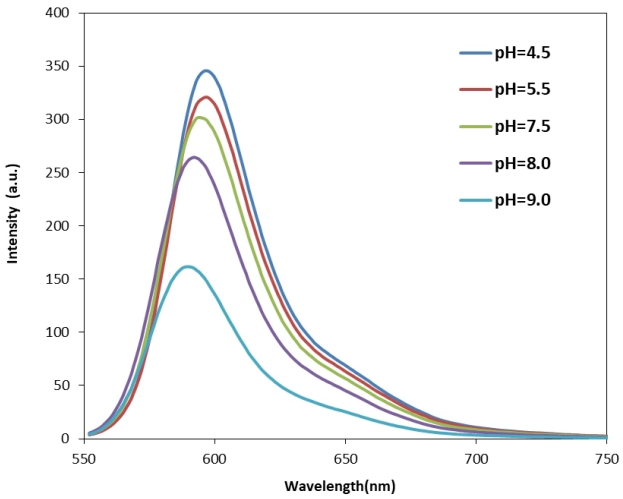

Figure 1. The pH dependent Emission spectra of Protonex™ Red 600.

DISCLAIMER

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