Protonex™ Green 500 Dextran

Catalog number: 21217
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21217)
Protonex™ Green 500 Dextran	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Protonex™ Green dye demonstrated pH-dependent fluorescence. Unlike most of the existing fluorescent dyes that are more fluorescent at higher pH, acidic conditions enhance the fluorescence of Protonex™ Green dye. The fluorescence of Protonex™ Green dye increases as pH decreases from neutral to the acidic. The lack of fluorescence outside the cell eliminates the wash steps. Protonex™ Green dye provides a powerful tool to monitor acidic cell compartments such as endosomes and lysosomes. Protonex™ Green dye is non-fluorescent outside the cells, but fluoresces brightly green in acidic compartments (such as phagosomes, lysosomes and endosomes). This Protonex™ Green enables the specific detection of cellular acidic compartments with reduced signal variability and improved accuracy for imaging or flow applications. Protonex™ Green has the spectral properties similar to those of FITC, making the common filter set of FITC readily available to the assays of Protonex™ Green.

AT A GLANCE

Protocol Summary

1. Prepare cells in a growth medium
2. Replace the medium with Protonex™ Green Dextran loading solution (100 µL/well for 96-well plate)
3. Incubate at 37°C for 5–20 minutes
4. Read Fluorescence at Ex/Em = 443/505 nm

KEY PARAMETERS

Fluorescence microscope

Emission	443 nm (FITC-compatible)
Excitation	505 nm (FITC-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™ Green Dextran loading solution:

1. Prepare a 1mg/mL stock solution of Protonex™ Green 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™ Green 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™ Green 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™ Green 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 = 443/505 nm.

Notes:

- Protonex™ Green Dextran becomes bright green 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

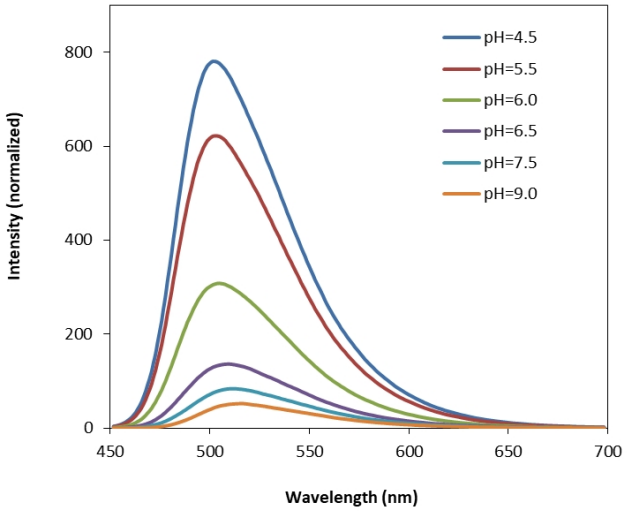

Figure 1. The pH dependent Emission spectra of Protonex™ Green 500.

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