Protonex™ Green 500 Dextran

Ordering Information	*Storage Conditions*
Product Number: 21217(1 mg)	Keep at -20 °C and avoid light

Introduction

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.

Chemical and Physical Properties

Molecular Weight: ~ 10,000
Solvent: Water
Spectral Properties: Ex/Em = 443/505 nm

Assay Protocol for Endocytosis

Brief Summary

Prepare cells in growth medium→ Replace the medium with Protonex™ Green Dextran loading solution (100 µL/well for 96-well plate)→ Incubate at 37°C for 5-20 minutes→ Wash and replace with HHBS →Read Fluorescence at Ex/Em= 443/505 nm

Note: The following is the recommended protocol for standard cell load. The protocol only provides a guideline, should be modified according to the specific needs.

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

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare RatioWorks™ Protonex™ Green Dextran loading solution:

2.1 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 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

2.2 Prepare a 20-100ug/mL Protonex™ Green Dextran loading solution in HHBS.

3. Run Endocytosis Assay

3.1 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).
Note1: 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.
Note2: 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.

3.2 Incubate the dye-loading plate at cell incubator for 5 to 20 minutes.

3.3 Wash and replace the dye-loading solution with HHBS or growth medium.

3.4 Run the endocytosis assay by monitoring the fluorescence at Ex/Em = 443/505 nm.

***Note**: The fluorescence signal from Protonex™ Green dextran is stable for at least one hour after trafficking to lysosomes has occurred. Because lysosomes have a lower pH compared to endosomes, the signal from Protonex™ Green dextran within the lysosomes is brighter than the signal from Protonex™ Green dextran within the endosomes. The lysosomal Protonex™ Green dextran concentration is directly dependent on endocytotic uptake; therefore, the modulation of endocytosis can be inferred from the intensity of Protonex™ Green dextran signal from the lysosomes.*

Ordering:sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support:support@aatbio.com; Tel: 408-733-1055

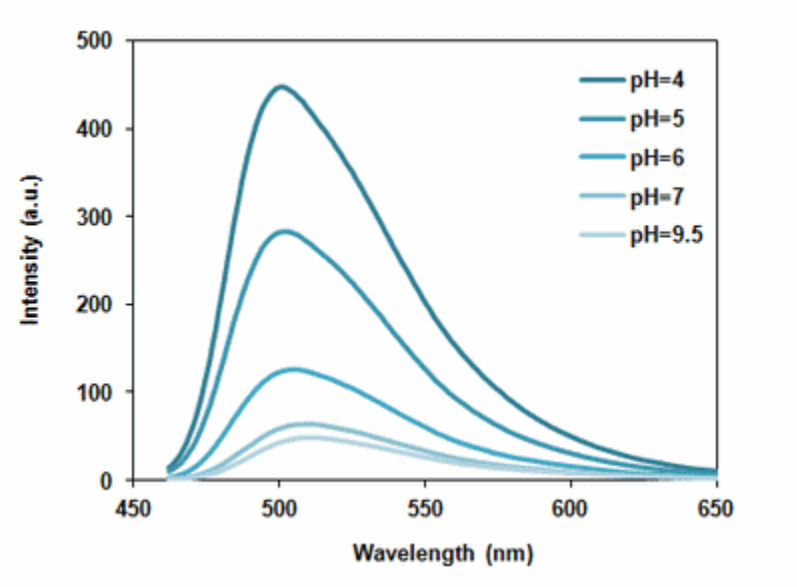

Figure 1. The fluorescence emission spectra of the Protonex™ Green Dextran.

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