# Protonex™Green 500-Latex Bead Conjugate

Catalog number: 21223
Unit size: 1 mL

| Component | Storage | Amount (Cat No. 21223) |
|---|---|---|
| Protonex™Green 500-Latex Bead Conjugate | Refrigerated (2-8 °C), Minimize light exposure | 1 mL |

## OVERVIEW

The Protonex™ Green 500-Latex Bead Conjugate is a ready-to-use reagent designed to study phagocytosis and phagosome acidification in live cells. This conjugate combines synthetic latex beads with Protonex™ Green 500, a novel pH-sensitive fluorophore that remains non-fluorescent at neutral pH and becomes highly fluorescent upon entering acidic environments such as maturing phagosomes and phagolysosomes.

As a standalone reagent, it enables users to integrate phagocytic detection into their own custom assays. The FITC-like excitation/emission properties of Protonex™ Green 500 make it compatible with a wide range of fluorescence imaging and detection systems. These conjugates can be used in combination with red fluorescent dyes like RFP, Calbryte™ 630 calcium dye, calcein red, or Cy5-labeled antibodies for multiplexed cell functional analysis. It is ideal for immunological research, drug discovery, and mechanistic studies of innate immune function, autophagy, or particle uptake.

## AT A GLANCE

**Chemical and Physical Properties**

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|---|---|
| **Solvent:** | Water |
| **Solids Content:** | 1% in PBS |
| **Number of Microspheres per mL:** | ~4e+10 |
| **Ex/Em:** | 445/503 nm |
| **Mean Diameter:** | 0.72 µm |

## KEY PARAMETERS

**Fluorescence microscope**

| | |
|---|---|
| Emission | FITC filter set |
| Excitation | FITC filter set |
| Recommended plate | Black wall/clear bottom |

## SAMPLE EXPERIMENTAL PROTOCOL

**Important**

The following is a recommended protocol for granulocytes. This protocol only provides a guideline and should be modified according to your specific experimental conditions.

**Protocol**

1. Prepare cells as desired. For example, prepare the granulocytes at $10^7$ cells/mL with Hanks and 20 mM Hepes buffer (HHBS), and add 100 µL to a polypropylene tube.

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

2. Add 1-10 µL of the Protonex™ Green 500-Latex Bead Conjugate to the tube and incubate with gentle shaking for 30 minutes at 37˚C.

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

3. Prepare an identical sample that is incubated at 4˚C and label it as a control.

4. At the end of the 30-minute incubation, stop phagocytosis by adding 2mL of ice-cold HHBS and mix well.

5. Wash the cells 2 times with cold HBSS.

6. Resuspend the cells in 500 µL of cold HBSS, keep the samples at 4˚C, and analyze immediately using a fluorescence microscope equipped with a FITC filter set.

   **Note:** For fluorescence microplate readers, monitor the fluorescence intensity at Ex/Em = 450/510 nm (Cutoff = 490 nm).

## DISCLAIMER