# Protonex™ Green 500-Zymosan A Conjugate

Catalog number: 21175
Unit size: 100 Tests

| Component | Storage | Amount (Cat No. 21175) |
|---|---|---|
| Protonex™Green 500-Zymosan Beads Conjugate | Refrigerated (2-8 °C), Minimize light exposure | 1 mL |

## OVERVIEW

The Protonex™ Green 500-Zymosan A Conjugate is a ready-to-use reagent designed to study phagocytosis and phagosome acidification in live cells. This conjugate combines Zymosan A particles, a biologically active yeast cell wall component 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

1. Plate the cells.
2. Treat cells with test compounds.
3. Add Protonex Dye Zymosan A conjugates in medium.
4. Incubate at 37°C for 60 minutes.
5. Monitor fluorescence by microscope or fluorescence plate reader.

## KEY PARAMETERS

**Fluorescence microscope**

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

**Fluorescence microplate reader**

| | |
|---|---|
| Cutoff | |
| Emission | 530nm |
| Excitation | 460nm |
| Recommended plate | Black wall/clear bottom |
| Instrument specification(s) | Bottom read mode |

## CELL PREPARATION

For guidelines on cell sample preparation, please visit:

https://www.aatbio.com/resources/guides/cell-sample-preparation.html

**Preparing Adherent Cells**

1. Plate cells overnight in a growth medium at 20,000-50,000 cells/well/100 µL in a 96-well plate.

**Note:** For RAW 264.7 cells used in this assay, we recommend plating 50,000 cells per well in 100 µL of medium in a 96-well plate and incubating them overnight. It is important to optimize the cell density for each cell line individually.

**Note:** Higher background fluorescence levels may be seen with poly-D-lysine coated microplates.

## SAMPLE EXPERIMENTAL PROTOCOL

**Treatment of cells:**

Add phagocytosis inhibitor or inducer (e.g., Cytochalasin D or LPS) at the desired concentrations. You may need to add vehicle controls to untreated wells. (For example: 11X working solution can be prepared in PBS, and 10 µL can be added to each well.)
**Note:** The time and concentration of phagocytosis effectors varies with cell types.

**Adding the Fluorescent Zymosan A conjugate:**

1. Add the suspension of Zymosan A conjugate to the cell culture microplate in a 1:10 dilution, or 10 µL of particles added to 100 µL of cell culture medium, and mix well.
2. Place the cells at 37°C for 60 minutes to 3 hours.

**Fluorescence Measurements:**

1. Wash the cells 2-3 times with HHBS Buffer (AAT Cat# 20011) or buffer of your choice.
2. Add 100 µL HHBS Buffer to each well.
3. Observe plate with a fluorescence microscope using the following filter set or read plate in a fluorescence plate reader with bottom read mode.

## EXAMPLE DATA ANALYSIS AND FIGURES

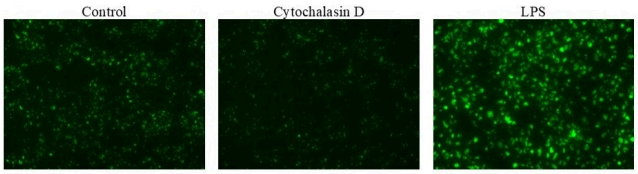


**Figure 1.** Examination of phagocytosis in RAW 264.7 cells Protonex™ Green 500-Zymosan A Conjugate (Cat #21175). RAW 264.7 cells were incubated with Cytochalasin D (to inhibit phagocytosis) or LPS (to induce phagocytosis) followed by incubation with Protonex™ Green 500-Zymosan conjugate for 60 minutes. The images were acquired using Keyence fluorescence microscopy.

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