Protonex™ Red 600-Zymosan A Conjugate

Catalog number: 21177
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21177)
Protonex™ Red 600 - Zymosan Beads Conjugate	Refrigerated (2-8 °C), Minimize light exposure	1 mL

OVERVIEW

The Protonex™ Red 600–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™ Red 600, 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 TRITC-like excitation/emission properties of Protonex™ Red 600 make it compatible with a wide range of fluorescence imaging and detection systems. These conjugates can be combined with green fluorescent dyes such as GFP, Calbryte™ 520, calcein AM, or FITC-labeled antibodies to enable multiplexed analysis of cell function and viability. 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	TRITC
Excitation	TRITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	
Emission	540nm
Excitation	590nm
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

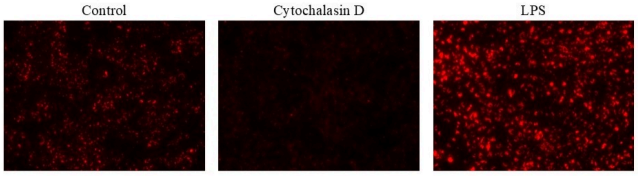

Figure 1. Examination of phagocytosis in RAW 264.7 cells using Protonex™ Red 600-Zymosan A Conjugate (Cat #21177). RAW 264.7 cells were incubated with Cytochalasin D (to inhibit phagocytosis) or LPS (to induce phagocytosis) followed by incubation with Protonex™ Red 600-Zymosan conjugate for 60 minutes. The images were acquired using Keyence fluorescence microscopy.

DISCLAIMER

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