Protonex™ Green 500-E. coli Conjugate

Catalog number: 21174
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21174)
Protonex™ Green 500-E. coli Conjugate	Refrigerated (2-8 °C), Minimize light exposure	100 Tests

OVERVIEW

The Protonex™ Green 500-E. coli Conjugate is a ready-to-use reagent designed to study phagocytosis and phagosome acidification in live cells. This conjugate combines E. coli particles, a biologically relevant model of bacterial uptake, 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 bacterial clearance.

AT A GLANCE

1. Plate the cells.
2. Treat cells with test compounds.
3. Add Protonex Dye E. coli 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 (e.g., Cytochalasin D) 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 E. coli Cojugate

1. Add the suspension of E. coli 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

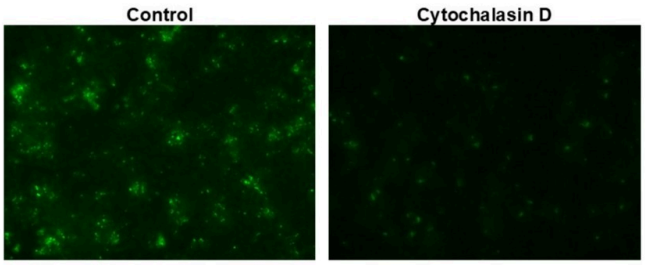

Figure 1. Examination of phagocytosis in RAW 264.7 cells using Protonex™ Green 500-E. coli Conjugate (Cat #21174). RAW 264.7 cells were incubated with Cytochalasin D (to inhibit phagocytosis) followed by incubation with Protonex™ Green 500-E. coli Conjugate for 60 minutes. The images were acquired using Keyence fluorescence microscope.

DISCLAIMER

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