

## Protonex™ Red 600-E. coli Conjugate

Catalog number: 21176  
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21176)
Protonex™ Red 600-E. coli Conjugate	Refrigerated (2-8 °C), Minimize light exposure	100 Tests

### OVERVIEW

The Protonex™ Red 600-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™ 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 used in combination with green fluorescent dyes like GFP, Calbryte™ 520, calcein AM, or FITC-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	TRITC
Excitation	TRITC
Recommended plate	Black wall/clear bottom

#### Fluorescence microplate reader

Cutoff	
Emission	590nm
Excitation	540nm
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.

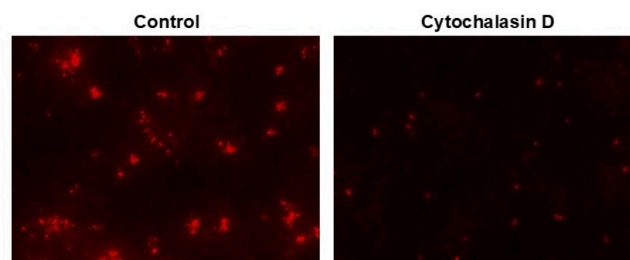
#### Adding the Fluorescent E. coli Cojugate

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™ Red 600-E. coli Conjugate (Cat #21176). RAW 264.7 cells were incubated with Cytochalasin D (to inhibit phagocytosis) followed by incubation with Protonex™ Red 600-E. coli Conjugate for 60 minutes. The images were acquired using Keyence fluorescence microscope.

### DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its

components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.