Cell Meter™ Fluorimetric Phagocytosis Assay Kit *Deep Red Fluorescence*

Catalog number: 21233
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21233)
Component A: Protonex™ Red 670- Zymosan Beads Conjugate	Refrigerated (2-8 °C), Minimize light exposure	150 µL
Component B: CytoTrace™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial (lyophilized)
Component C: DMSO	Refrigerated (2-8 °C)	100 µL

OVERVIEW

The Cell Meter™ Fluorimetric Phagocytosis Assay Kit (Deep red fluorescence) is designed to evaluate phagocytic activity in live cells. It is cellular process in which cells engulf and digest particles, such as pathogens or cellular debris, to maintain homeostasis. It features a ready-to-use suspension of Zymosan particles conjugated to Protonex™ Red 670, a novel pH-sensitive dye. Unlike traditional fluorophores, rotonex™ Red 670 is non-fluorescent at neutral pH, becoming highly fluorescent only upon acidification making it ideal for monitoring internalization and lysosomal fusion during phagocytosis. Once engulfed, the Zymosan conjugates are trafficked into acidic phagosomes/phagolysosomes, where the rotonex™ Red 670 dye is activated. The spectral property of the dye is similar to that of Cy5, allowing for seamless integration into existing Cy5-compatible detection workflows.

The kit also contains a green fluorescent viability dye to enable multiplexed analysis of live cells and phagocytosis within the same assay. This two-color system provides a powerful and versatile tool for studying the dynamics and regulation of phagocytosis in various biological and immunological contexts. Suitable for applications in immunology, drug screening, and cellular function research, the assay is compatible with fluorescence microscopy, flow cytometry, and microplate-based formats.

AT A GLANCE

Protocol Summary

1. Plate cells.
2. Add Protonex™ Red 670-Zymosan Beads Conjugate solution.
3. Incubate at 37 °C for 60 minutes.
4. Add CytoTrace™ Green.
5. Incubate at 37 °C for 30 minutes.
6. Monitor fluorescence by microscopy using Cy5 and FITC filters.

Important Note

Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy5/FITC filters

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 the 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.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

CytoTrace™ Green Stock Solution (400X)

1. Add 20 µL of DMSO (Component C) to the vial of CytoTrace™ Green (Component B) and mix thoroughly.

   Note: Please prepare the stock solution before use. Unused 400X CytoTrace™ Red stock solution in DMSO can be aliquoted into single-use vials and stored at -20 °C, protected from light

PREPARATION OF WORKING SOLUTION

Protonex™ Red 670- Zymosan Beads Conjugate Solution (12X)

1. Add 150 µL of Protonex™ Red 670- Zymosan Beads Conjugates (Component A) to 1.5mL of cell growth medium (containing 10% FBS), and mix well.

   Note: Unused beads can be stored at 4 °C.

CytoTrace™ Green Working Solution (12X)

1. Add 5 µL of CytoTrace™ Green stock solution (400X) to 2 mL of cell growth medium and mix well.

   Note: Please prepare the stock solution before use.

SAMPLE EXPERIMENTAL PROTOCOL

Phagocytosis Assay Protocol

1. For negative control, add 25 µL of 6X Cytochalasin D (not provided). The final concentration in the well should be 10 µM.

Similarly, for positive control, add LPS to a final concentration of 250ng/mL. Prepare your test compounds and add to the wells.

Note: To prepare a 6X Cytochalasin D solution, add 18 µL of 10 mM Cytochalasin D (not included) to 3 mL of cell growth medium, and mix thoroughly. Cytochalasin D and LPS concentrations need to be optimized for each specific cell line.

Note: Cytochalasin D and LPS are not provided in the kit.

2. Incubate the plate in the cell incubator for 30 minutes for Cytochalasin and for 8 hours or more for LPS treatment.

3. Add 12.5 µL of the Protonex™ Red 670-Zymosan Beads Conjugate Solution to each well.

4. Incubate the plate in a cell incubator for 60 minutes.

   Note: The incubation time should be optimized by users for each individual cell lines.

5. Add 12.5 µL of the 12X CytoTrace™ Green working solution to each well.

6. Incubate the plate in a cell incubator for 30 minutes.

7. Wash the plate twice with 1X PBS.

8. Observe phagocytosis inside the cells with Cy5 filter (Ex/Em = 640/680 nm) for deep red fluorescence and CytoTrace™ Green with FITC filter (Ex/Em = 490/525 nm) for green fluorescence.

EXAMPLE DATA ANALYSIS AND FIGURES

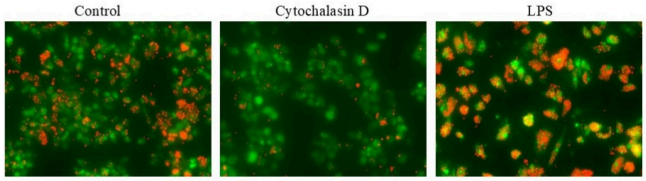

Figure 1. Examination of phagocytosis in RAW 264.7 cells using Cell Meter™ Fluorimetric Phagocytosis Assay Kit (Cat# 21233). RAW 264.7 cells were incubated with Cytochalasin D (to inhibit phagocytosis) or LPS (to induce phagocytosis) followed by incubation with Protonex™ Red 670-Zymosan Beads in growth medium for 60 minutes and then stained with CytoTrace™ Green for 30 minutes. The images were acquired using Keyence Fluorescence microscopy.

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