Cell Meter™ Fluorimetric Phagocytosis Assay Kit *Red Fluorescence*

Catalog number: 21225
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21225)
Component A: Protonex™ Red 600-Latex Bead Conjugates	Refrigerated (2-8 °C), Minimize light exposure	1 vial (15 µL)
Component B: CytoTrace™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)

OVERVIEW

Phagocytosis is a cellular process in which particles are internalized within a plasma membrane-derived vesicle. This mechanism is fundamental to the innate immune response, facilitating the recognition, engulfment, and degradation of pathogens. Additionally, phagocytosis plays a key role in tissue homeostasis and remodeling by mediating the clearance of apoptotic cells. The uptake of particles via phagocytosis is influenced by factors such as particle size, receptor-ligand interactions, and cytoskeletal rearrangements. Following internalization, phagosomes undergo maturation and fuse with lysosomes to form phagolysosomes, where enzymatic degradation occurs in an increasingly acidic environment. The Cell Meter™ Fluorimetric Phagocytosis Assay Kit incorporates Protonex™ Red 600-latex bead conjugates, which are pH-sensitive and supplied as a ready-to-use suspension. Unlike many traditional fluorescent probes, these bead conjugates are non-fluorescent outside of the cell but exhibit a significant increase in fluorescence upon acidification within phagosomes and phagolysosomes. This unique property eliminates the need for a trypan blue quenching step, enhancing the efficiency of the assay and making it an ideal tool for studying phagocytic activity and its regulation. The kit also includes a green fluorescent cell viability dye, allowing for the concurrent assessment of cell viability and phagocytosis via fluorescence microscopy. Additionally, the assay is compatible with fluorescence microplate readers and flow cytometry, offering flexibility in detection methods and high-throughput capabilities for quantitative analysis.

AT A GLANCE

Protocol Summary

1. Plate cells.
2. Add 12.5 µL Protonex™ Red 600-Latex Bead Conjugates.
3. Incubate at 37 °C for 4 hours.
4. Add 12.5 µL CytoTrace™ Green.
5. Incubate at 37 °C for 30 minutes.
6. Monitor fluorescence by microscopy using Texas Red 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)	Texas Red/FITC filter

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

Protonex™ Red 600-Latex Bead Conjugate Solution (12X)

1. Add 8 µL of Protonex™ Red 600-Latex Bead Conjugates (Component A) to 2 mL of cell growth medium (containing 10% FBS), and mix well.

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

CytoTrace™ Green Stock Solution (400X)

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

   Note: Unused CytoTrace™ Green DMSO stock solution can be aliquoted into single-use vials and stored at -20 °C, protected from light.

PREPARATION OF WORKING SOLUTION

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.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 25 µL of 6X Cytochalasin D (not provided) as a positive control or your test compound into each well. The final concentration in the well should be 10 µM.

   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. Be sure to optimize the Cytochalasin D concentration for each specific cell line used in the assay.

2. Incubate the plate in the cell incubator for 30 minutes.

3. Add 12.5 µL of the 12X Protonex™ Red 600-Latex Bead Conjugate solution to each well.

4. Incubate the plate in the cell incubator for 4 hours.

   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 the cell incubator for 30 minutes.

7. Wash the plate twice with 1X PBS.

8. Observe phagocytosis inside the cells with Texas Red filter (Ex/Em = 570/600 nm) and CytoTrace™ Green with FITC filter (Ex/Em = 490/525 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

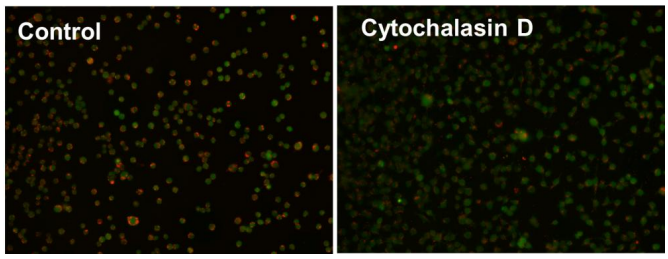

Figure 1. Examination of phagocytosis in RAW 264.7 cells using Cell Meter™ Fluorimetric Phagocytosis Assay Kit (Cat# 21225). RAW 264.7 cells were incubated with (B) or without (A) Cytochalasin D for 30 min before Protonex™ Red 600-Latex Beads in the growth medium was added and incubated for 4 hours before Cell Tracker was added and incubated for 30 minutes. The images were taken using Keyence Fluorescence microscopy.

DISCLAIMER

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