Cell Meter™ Fluorimetric Phagocytosis Assay Kit *Red Fluorescence*

Catalog number: 21225
Unit size: 100 Tests

Component	Storage	Amount
Component A: Protonex 600 Red-Latex Beads Conjugate	Refrigerated (2-8 °C), Minimize light exposure	1 vial (15 µL)
Component B: CytoTrace™ Green	Minimize light exposure, Refrigerated (2-8 °C)	1 vial
Component C: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)

OVERVIEW

Phagocytosis is defined as the cellular uptake of particles within a plasma-membrane envelope by a cell. The process of phagocytosis is critical in the innate immune response by engulfment and destruction of invading microorganisms. Phagocytosis is also required in maintaining tissue homeostasis and remodeling by the clearance of apoptotic bodies. The uptake mechanism of phagocytosis depends on the size of the particles, receptor-ligand interactions, and involvement of the cytoskeleton. Once internalized, the phagosome fuses with lysosomes to form secondary phagolysosome for digestion, resulting in progressive decrease of pH. Our Cell Meter™ Fluorimetric Phagocytosis Assay Kit utilizes a unique pH dependent Protonex™ 600 Red-latex bead conjugates. The beads are in ready to use suspension. Unlike most of the existing fluorescent dyes, the Protonex™ 600 Red-latex bead conjugate is non-fluorescent outside of the cells. However, its fluorescence dramatically increases as they are inside the acidic phagosomes/phagolysosomes. This characteristic makes it easy to use without trypan blue quenching step and a robust tool to study phagocytosis and its regulations. Cell Meter™ Fluorimetric Phagocytosis Assay Kit also includes a green fluorescent cell viability dye, which allowing the simultaneous detection of both live cells and the process of phagocytosis by fluorescent microscopy. This assay can also be adapted for fluorescence micro-plate reader and flow cytometry detections.

AT A GLANCE

Protocol Summary

1. Plate cells
2. Add 12.5 µL Protonex™ 600 Red-Latex Beads Conjugate
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 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

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.

1. Protonex™ 600 Red-Latex Beads Conjugate solution (12X)
Add 8 µL of Protonex™ 600 RedLatex Beads Conjugate (Component A) in 2 mL cell growth medium (containing 10% FBS).

Note The unused beads can be stored at 4 °C.

2. CytoTrace™ Green stock solution (400X)
Add 20 µL of DMSO (Component C) into the vial of CytoTrace™ Green (Component B) and mix them well.

Note The unused CytoTrace™ Green DMSO stock solution can be aliquoted into single use vials and stored at -20 °C.

PREPARATION OF WORKING SOLUTION

CytoTrace™ Green working solution (12X)
Add 5 µL of CytoTrace™ Green stock solution (400X) in 2 mL of cell growth medium and mix them well.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 25 µL of 6X Cytochalasin D as a positive control or your test compound into each well.

   Note The concentration of Cytochalasin D used in the assay should be optimized for each individual cell line.

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

3. Add 12.5 µL of Protonex™ 600 Red-Latex Beads Conjugate solution (12X) into 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 CytoTrace™ Green working solution (12X) 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

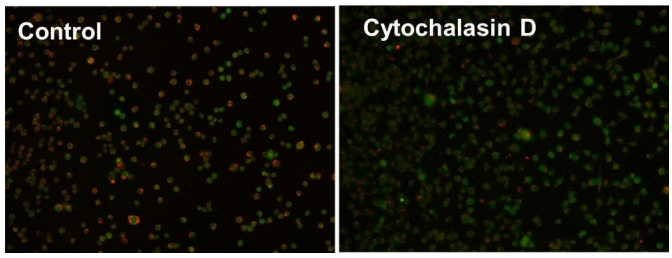

Figure 1.
Examination of phagocytosis in RAW 264.7 cells using Cell Meter™ Fluorimetric Phagocytosis Assay Kit (Cat# 21225). RAW 264.7 cells were incubated with (B) or without (A) Cytochalasin D for 30 min before Protonex™ 600 Red-Latex Beads in growth medium was added and incubated for 4 hours before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

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