Cell Meter™ Fluorimetric Phagocytosis Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 21225 (100 tests)	Keep in refrigerator Avoid exposure to light	Fluorescent Microscopy

Introduction

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.

Kit Components

Components	Amount
Component A: Protonex 600 Red-Latex Beads Conjugate	15 µL
Component B: CytoTrace™ Green	1 vial (lyophilized)
Component C: DMSO	100 µL

Note: *Cytochalasin D (Sigma Cat# C2618) used in the assay is not provided, please purchase it from Sigma directly.*

Assay Protocol for One 96-well Plate

Brief Summary

Plate cells → Add Protonex™ 600 Red-Latex Beads Conjugate → Incubate at 37 °C (4 hours)→ Add CytoTrace™ Green → Incubate at 37 °C (30 minutes)→ Monitor fluorescence by microscopy

Note: Equilibrate all the kit components to room temperature before starting the experiment.

1. **Prepare cells:**

   For adherent cells: Plate cells overnight in growth medium at 20,000-50,000 cells/well/100 µL for a 96-well plate.

   Note 1: Each cell line should be evaluated on the individual basis to determine the optimal cell density.
   Note 2: For RAW 264.7 cells that we used in this assay we recommend to plate cells at 50,000 cells/well/100 µL in 96-well plate for overnight.

2. **Prepare stock solutions:**

   2.1. Prepare 6X Cytochalasin D: Add 18 µL of 10 mM Cytochalasin D (final concentration in the well is 10 µM) or 18 µL of DMSO in 3 mL cell growth medium.

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

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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

2.3. Prepare 12X CytoTrace™ Green: Add 20 µL of DMSO (Component C) into the vial of CytoTrace™ Green (Component B) to make 400X stock solution. And then add 5 µL in 2 mL cell growth medium to have 12X CytoTrace™ Green solution
Note: The unused CytoTrace™ Green *DMSO stock solution can be aliquoted into single use vials and stored at -20°C.*

3. Phagocytosis assay protocol:

3.1. Add 25 µL of 6X Cytochalasin D (from step 2.1) or DMSO solution (from step 2.1) into each well.
Note: The concentration of Cytochalasin D used in the assay should be optimized for each individual cell line.

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

3.3. Add 12.5 µL of 12X Protonex™ 600 Red-Latex Beads Conjugate solution (from step 2.2) into each well.

3.4. Incubate the plate in the cell incubator for 4 hours.
Note: The incubation time should be optimized by users for each individual cell lines.

3.5. Add 12.5 µL of 12X Cell Tracker (from step 2.3) into each well.

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

3.7. Wash the plate twice with 1XPBS.

4. 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)

Data Analysis

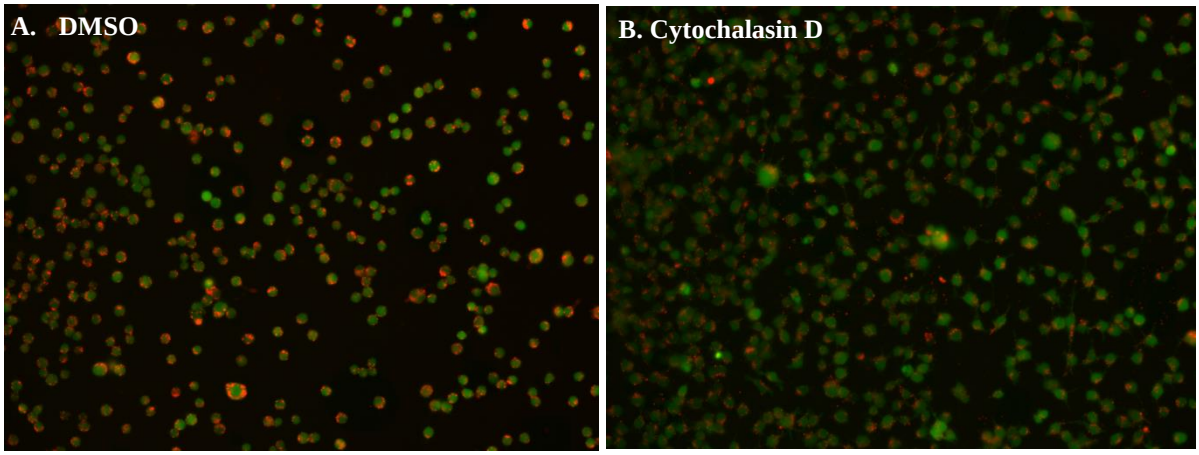

Figure1: RAW 264.7 cells were incubated with (B) or without (A) Cytochalasin D for 30 min before Protonex™ 600 Red-Latex Beads in growth medium was added and incubated for 4 hours before CytoTrace™ Green was added and incubated for 30 minutes. The images were taken using Keyence Fluorescence croscopy.

References

1. Flannagan RS, Jaumouillé V, Grinstein S. The cell biology of phagocytosis. Annu Rev Pathol. 2012;7:61-98.
2. Gordon S. Phagocytosis: An Immunobiologic Process. Immunity. 2016 Mar 15; 44(3):463-75.