Product Manual

CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell Substrate)

Catalog Number

MBS168645 **96 assays**

Introduction

In mammals, phagocytosis by phagocytes (e.g., macrophages, dendritic cells, and neutrophils) is essential for a variety of biological events, including tissue remodeling and the continuous clearance of dying cells. Furthermore, phagocytosis represents an early and crucial event in triggering host defenses against invading pathogens. Phagocytosis comprises a series of events, starting with the binding and recognition of particles by cell surface receptors, followed by the formation of actin-rich membrane extensions around the particle. Fusion of the membrane extensions results in phagosome formation, which precedes phagosome maturation into a phagolysosome. Pathogens inside the phagolysosome are destroyed by lowered pH, hydrolysis, and radical attack (Figure 1). These early events that are mediated by the innate immune system are critical for host survival. As a result of this process, pathogen-derived molecules can be presented at the cell surface (antigen presentation), allowing the induction of acquired immunity.

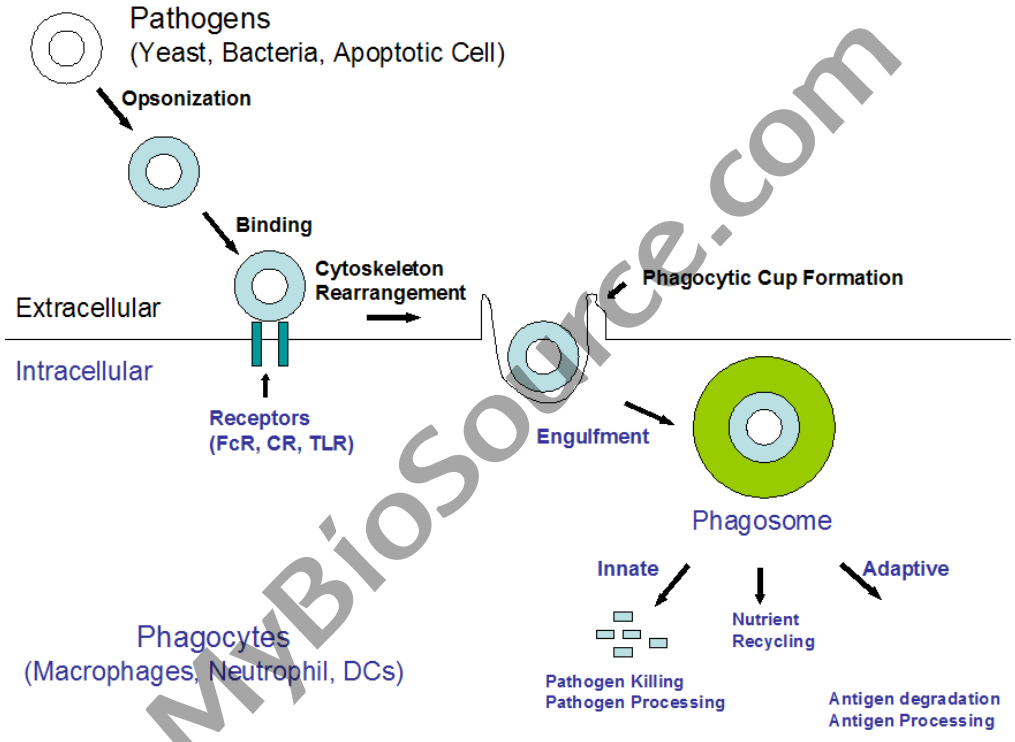


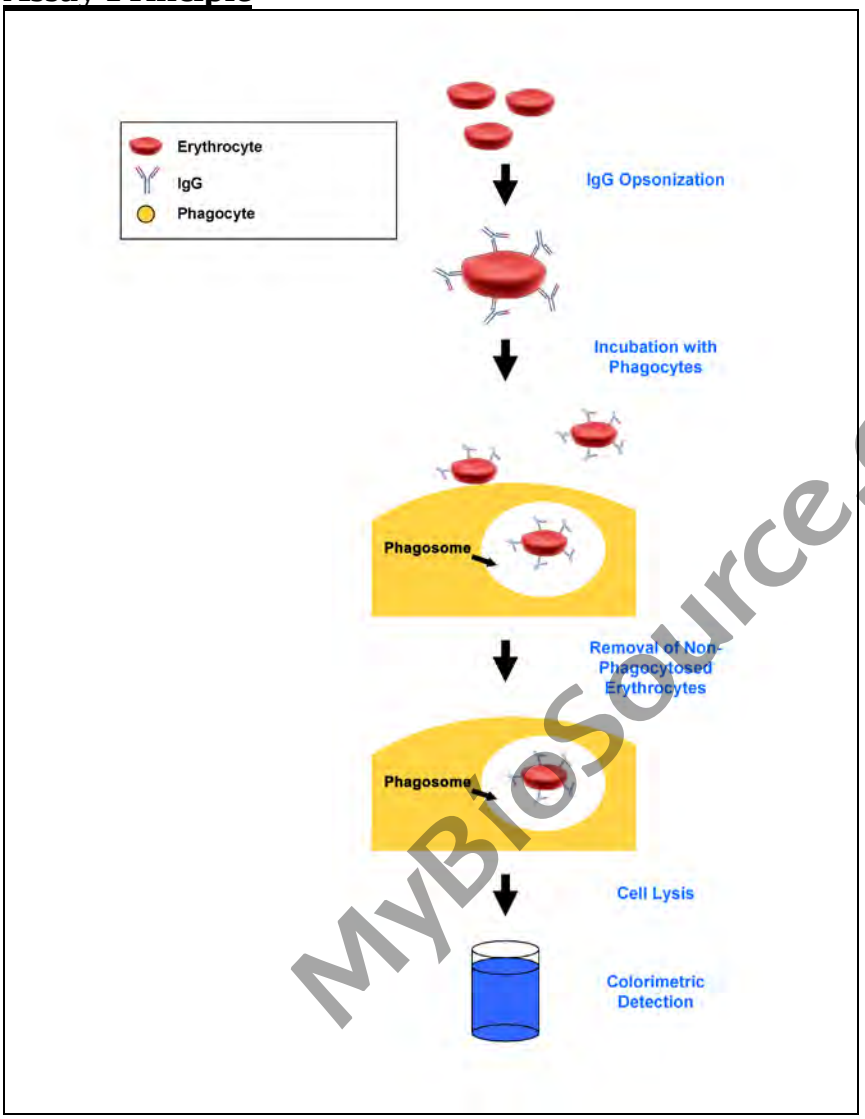
Figure 1: Phagocytosis Processes.

Traditionally, erythrocytes (red blood cells) are commonly used in phagocytosis assay. For FcR mediated phagocytosis, erythrocytes are first opsonized with serum or IgG before they are added to phagocytes. After removal of non-phagocytic erythrocytes, engulfed erythrocytes per phagocyte cell are manually counted (expressed as phagocytosis index or engulfed erythrocytes per phagocyte). This manual counting method is quite cumbersome, time-consuming, and difficult when testing a large number of samples.

CytoSelect™ 96-well Phagocytosis Assay does **not** involve subjective manual counting of erythrocytes. Instead cells are lyzed and detected by the proprietary erythrocyte substrate in a microtiter plate reader (Figure 2). This format provides a quantitative, high-throughput method to

accurately measure phagocytosis. The CytoSelect™ 96-well Phagocytosis Assay provides a robust system for screening TLR ligands, phagocytosis activators or inhibitors. Each kit provides sufficient quantities to perform 96, 48, 24 tests in a 96, 48, 24-well plate, respectively.

Assay Principle



Related Products

1. : CytoSelect™ 96-Well Phagocytosis Assay (Zymosan, Colorimetric Format)
2. : CytoSelect™ Leukocyte-Endothelium Adhesion Assay
3. : CytoSelect™ Leukocyte-Epithelium Adhesion Assay
4. : CytoSelect™ Leukocyte Transmigration Assay

Kit Components

1. Opsonization Solution (Part No. 122001): One 20 µL tube of Rabbit Anti-Sheep RBC, IgG
2. 10X Wash Solution (Part No. 122002): One 10 mL bottle
3. Lysis Buffer A (Part No. 122003): One 20 mL bottle
4. Lysis Buffer B (Part No. 122004): One 150 µL tube
5. 100X Substrate Solution (Part No. 122005): One 150 µL amber tube
6. Phagocytosis Inhibitor (Part No. 122006): One 20 µL amber tube of 2 mM Cytochalasin D in DMSO

Materials Not Supplied

1. Live sheep erythrocytes (MP Biomedicals or Lampire Biological Laboratories)
2. Phagocytes and Culture Medium
3. 37ºC Incubator, 5% CO_2 Atmosphere
4. Light Microscope
5. 96-well Microtiter Plate Reader

Storage

Store all kit components at 4ºC.

Preparation of Reagents

- 10X Wash Solution: In a sterile tube, dilute the provided 10X Wash Solution in sterile, cell culture grade water. For example, to prepare a 10 mL solution, add 1 mL of 10X Wash Solution to 9 mL of sterile cell culture grade water. Store at 4 °C prior to use.
- Sheep Erythrocyte Opsonization: Resuspend live sheep erythrocytes at 1-5 x 10^8 cells/mL in sterile PBS. Add the Opsonization Solution to the sheep erythrocyte suspension at a 1:500 dilution. Mix well and incubate at 37ºC for 30 minutes. Wash twice with sterile PBS and resuspend at 1-5 x 10^8 cells/mL in sterile PBS or phagocyte culture medium. Use immediately and discard any unused solution.
- 1X Lysis Buffer: Prepare the appropriate volume for the number of samples being tested. Prior to using, dilute the provided Lysis Buffer B 1:150 in Lysis Buffer A. Do not store.
- 100X Substrate Solution: Prepare the appropriate volume for the number of samples being tested. Prior to using, dilute the provided 100X Substrate Solution 1:10 in Lysis Buffer. For example, to prepare a 1 mL solution, add 100 µL of 100X Substrate Solution to 900 µL of Lysis Buffer. Do not store.

  Note: This diluted substrate will be diluted once more, yielding a 1X final concentration.

Assay Protocol: Adherent Phagocytes

The following assay protocol is written for a 96-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats.

Culture Dish	96-well	48-well	24-well
Phagocyte Seeding Volume (µL/well)	100	300	500
IgG Opsonized RBC Suspension (µL/well)	10	30	50
1X Wash Solution (µL/well)	200	600	1000
1X PBS (µL/well)	200	600	1000
1X Lysis Buffer (µL/well)	120	240	360
Diluted Substrate Solution (µL/well)	10	20	30

Table 1: Dispensing Volumes of Different Plate Formats

1. Harvest and resuspend phagocytic cells in culture medium at $1 – 5 \times 10^5$ cells/mL or the appropriate concentration that yields 50-80% confluency after overnight incubation. Seed 100 µL in each well of a 96-well plate and incubate overnight at 37ºC, 5% CO_2.
2. Treat phagocytes with desired activators or inhibitors.
3. Add 10 µL of IgG opsonized erythrocyte suspension (see Preparation of Reagents Section) to each well. Mix well and immediately transfer the plate to a cell culture incubator for 15 minutes to 2 hours.
4. Remove the culture medium by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.
5. Add 200 µL of cold 1X Wash Solution to each well. **Incubate wells for 30 seconds on an orbital shaker.**
6. Promptly remove the Wash Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.
7. Wash once with 200 µL of cold 1X PBS.
8. Promptly remove the PBS solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.
9. Add 120 µL of 1X Lysis Buffer to each well. Pipette each well 5-10 times to mix thoroughly.
10. Transfer 90 µL of the mixture to a 96-well microtiter plate.
11. Add 10 µL of prediluted Substrate Solution to each well (see Preparation of Reagents Section). Pipette each well 5-10 times to ensure a homogeneous mixture. Incubate the plate for 10-20 minutes at room temperature.
12. Measure the absorbance at 610-630 nm in a 96-well microtiter plate reader.

Assay Protocol: Suspension Phagocytes

1. Harvest and resuspend phagocytic cells in culture medium at 0.2 – 1.0 x 10^6 cells/mL. Seed 100 µL in each well of a 96-well plate.
2. Treat phagocytes with desired activators or inhibitors.
3. Add 50 µL of IgG opsonized erythrocyte suspension (see Preparation of Reagents Section) to each well. Mix well and immediately transfer the plate to a cell culture incubator for 15 minutes – 2 hours.
4. Remove the culture medium by centrifugation and gentle aspiration.
5. Add 200 µL of cold 1X Wash Solution to each well. **Incubate wells for 30 seconds on an orbital shaker.**
6. Promptly remove the Wash Solution by centrifugation and gentle aspiration.
7. Wash once with 200 µL of cold 1X PBS.
8. Promptly remove the PBS by centrifugation and gentle aspiration.
9. Add 120 µL of 1X Lysis Buffer to each well. Pipette each well 5-10 times to mix thoroughly.
10. Transfer 90 µL of the mixture to a 96-well microtiter plate.
11. Add 10 µL of prediluted Substrate Solution to each well (see Preparation of Reagents Section). Pipette each well 5-10 times to ensure a homogeneous mixture. Incubate the plate for 10-20 minutes at room temperature.
12. Measure the absorbance at 610-630 nm in a 96-well microtiter plate reader.

Erythrocyte Standard Curve (Optional)

1. Harvest and resuspend live erythrocytes in sterile PBS at 1-2 x 10^8 cells/mL.
2. Dilute erythrocytes 10-fold with 1X Lysis Buffer to 1-2 x 10^7 cells/mL.
3. Prepare a 2-fold, serial dilution in 1X Lysis Buffer, including a Lysis Buffer blank.
4. Transfer 90 µL of each dilution to a 96-well microtiter plate.
5. Add 10 µL of prediluted Substrate Solution to each well (see Preparation of Reagents Section). Pipette each well 5-10 times to ensure a homogeneous mixture. Incubate the plate for 10-20 minutes at room temperature.
6. Measure the absorbance at 610-630 nm in a 96-well microtiter plate reader.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ 96-well Phagocytosis Assay Kit. Absorbance measurements were performed on a Microplate Autoreader EL311 (Bio-Tek Instruments Inc.) with a 630 nm filter. One should use the data below for reference only. This data should not be used to interpret actual results.

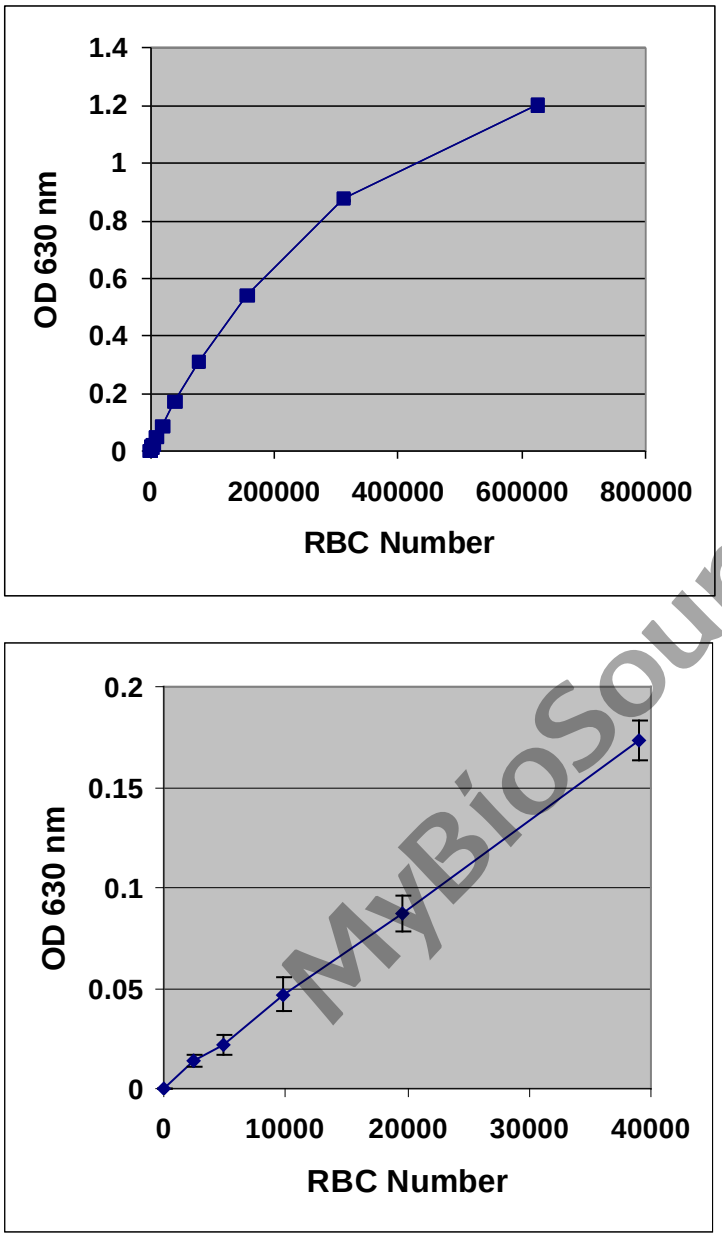
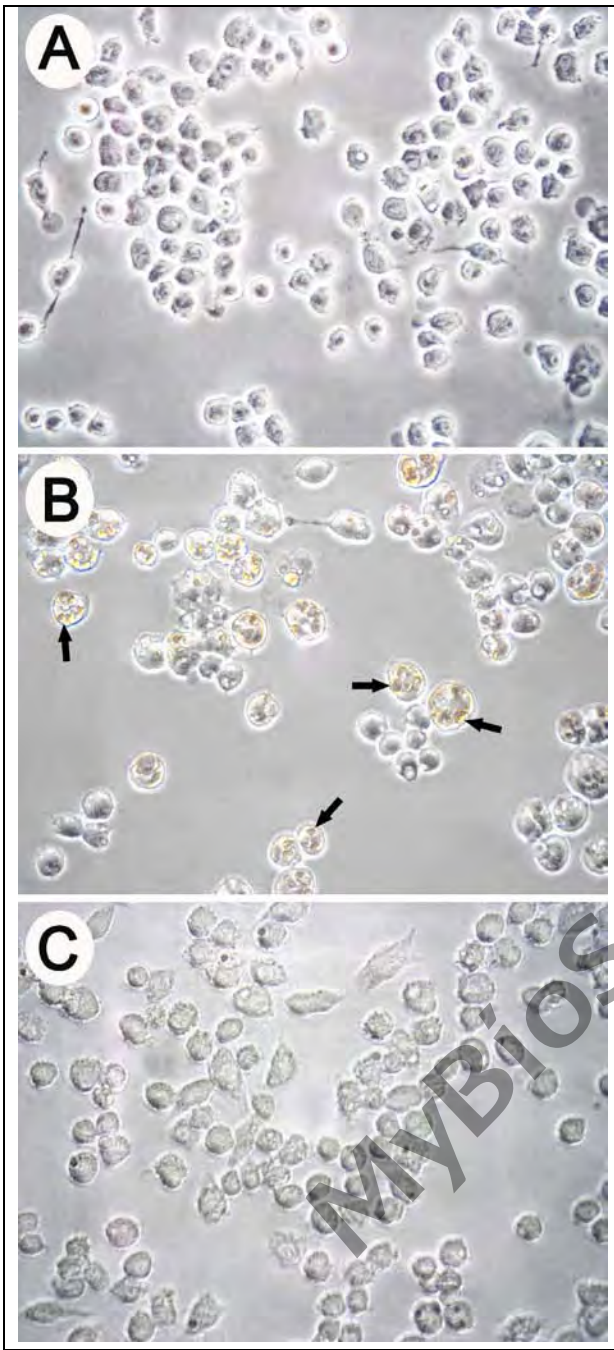


Figure 2. Sheep Red Blood Cell Dose Curve.



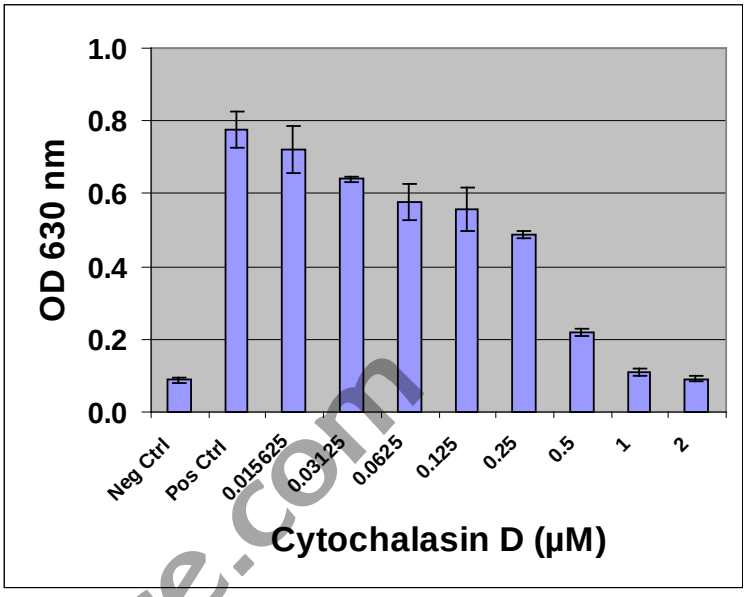


Figure 3: Inhibition of Raw 264.7 Macrophage Phagocytosis by Cytochalasin D. 20,000 cells/well of Raw 264.7 macrophages were seeded overnight in a 96-well plate. Cytochalasin D was used to pretreat Raw 264.7 cells for 1 hr at 37ºC before IgG opsonized sheep red blood cells were added at 50:1 ratio. Phagocytosis was stopped after 30 minutes and the amount of engulfed red blood cells was determined as described in the Assay Protocol. A: Non-opsonized RBC + Raw 264.7; B: IgG opsonized RBC + Raw 264.7; C: IgG opsonized RBC + Raw 264.7 (2 µM Cytochalasin D pretreated). Above: Cytochalasin D dose response.

References

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3. Janeway, C. A., Jr., and Medzhitov R. (2002) *Annu. Rev. Immunol.* **20**:197–216.
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Recent Product Citations

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2. Lee, J.K. et al. (2011). Regulator of G-protein signaling-10 negatively regulates NF-kB in microglia and neuroprotects dopaminergic neurons in hemiparkinsonian rats. *J. Neurosci.* **31**:11879-11888.
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