Product Manual

CytoSelect™ 96-Well Phagocytosis Assay (Zymosan Substrate)

Catalog Number

CBA-224	96 assays

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Not for use in diagnostic procedures

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Creating Solutions for Life Science Research

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.

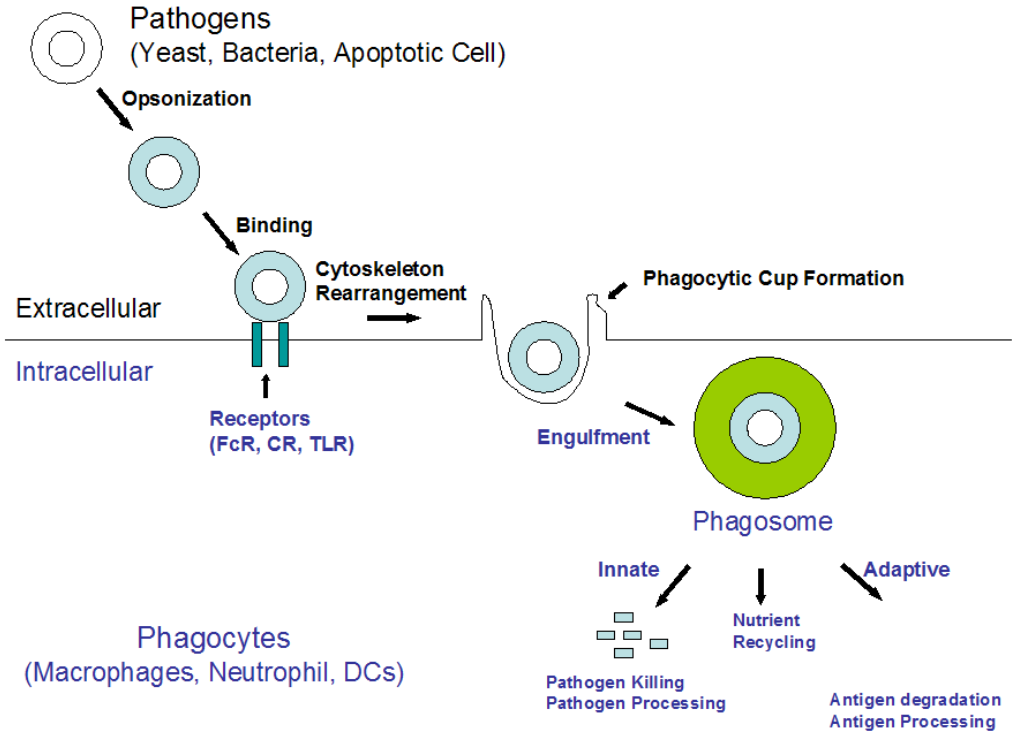


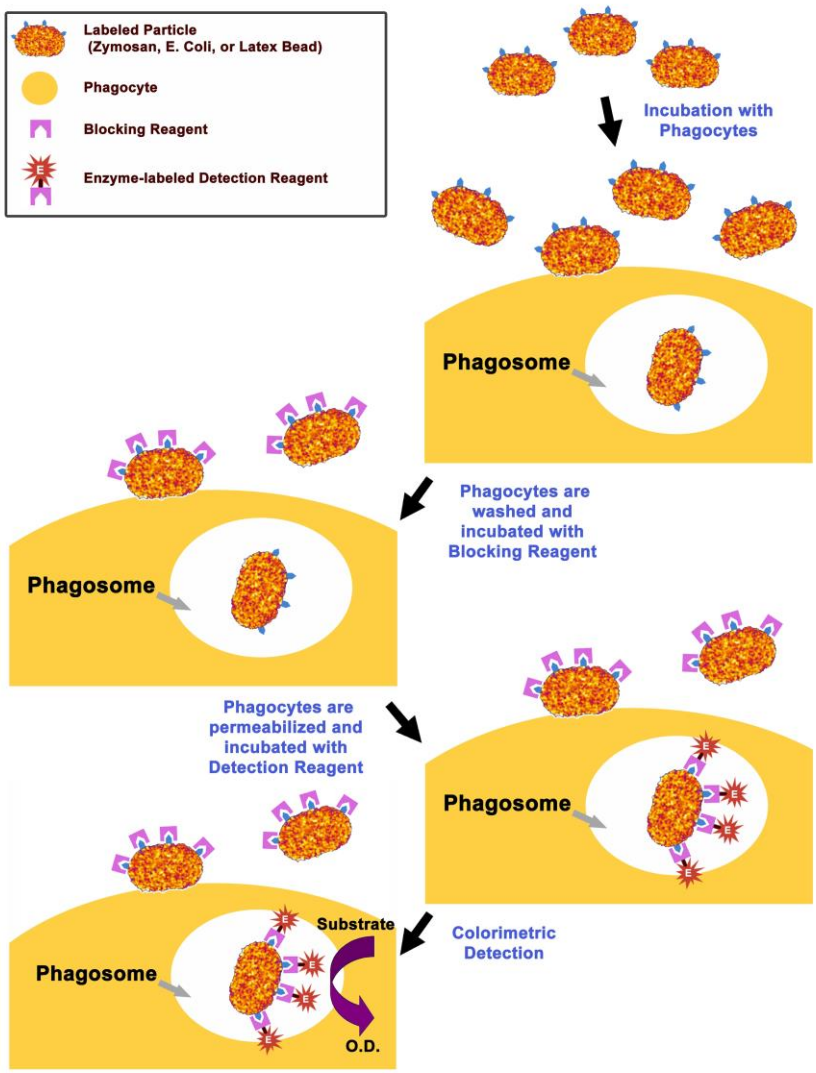
Figure 1: Phagocytosis Processes.

Zymosan (*Saccharomyces cerevisiae*) is prepared from yeast cell wall and consists of protein-carbohydrate complexes. Zymosan is a commonly used pathogen in phagocytosis assays. Typically, engulfed Zymosan particles are manually counted (expressed as a phagocytosis index or engulfed particles per phagocyte). This manual counting method is quite cumbersome, time-consuming, and difficult when testing a large number of samples.

Cell Biolabs' CytoSelect™ 96-well Phagocytosis Assay (Zymosan) uses prelabeled Zymosan particles as a phagocytosis pathogen; however, it does **not** involve subjective manual counting of Zymosan particles inside cells. Instead external Zymosan particles are blocked before the colorimetric detection of engulfed particles (Figure 2). This format provides a quantitative, high-throughput method to

accurately measure phagocytosis. The CytoSelect™ 96-well Phagocytosis Assay (Zymosan) 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. CBA-220: CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell, Colorimetric Format)
2. CBA-210: CytoSelect™ Leukocyte-Endothelium Adhesion Assay
3. CBA-211: CytoSelect™ Leukocyte-Epithelium Adhesion Assay
4. CBA-212: CytoSelect™ Leukocyte Transmigration Assay

Kit Components

1. Zymosan Suspension (Part No. 122401): One 1 mL tube of prelabeled Zymosan in PBS, 5 X 10^8 particles/mL
2. Fixation Solution (Part No. 122402): One 20 mL bottle of 3.2% Buffered Formaldehyde Solution
3. Blocking Reagent (100X) (Part No. 122403): One 200 µL tube
4. 10X Permeabilization Solution ((Part No. 122404): One 1.5 mL tube of PBS/1% Triton X-100
5. Detection Reagent (250X) (Part No. 122405): One 50 µL tube
6. Detection Buffer (Part No. 122406): One 10 mL bottle
7. Substrate (Part No. 122407): One 12 mL amber bottle
8. Stop Solution (Part No. 122408): One 12 mL bottle
9. Phagocytosis Inhibitor (Part No. 122006): One amber tube – 20 µL of 2 mM Cytochalasin D in DMSO

Materials Not Supplied

1. Adherent Phagocytes and Culture Medium
2. PBS, PBS/0.1% BSA
3. 37ºC Incubator, 5% CO_2 Atmosphere
4. Light Microscope
5. 96-well Microtiter Plate
6. 96-well Microtiter Plate Reader

Storage

Store all kit components at 4ºC.

Preparation of Reagents

- Zymosan Suspension: Thaw Zymosan suspension at 4ºC. Either nonopsonized or opsonized Zymosan particles can be used in phagocytosis assay. To opsonize Zymosan particles, incubate particles with desired serum or IgG for 30 minutes at 37ºC, pellet particles by centrifugation and wash a few times with sterile 1X PBS. Prior to using, resuspend the particles in the same volume of sterile 1X PBS. Store at 4ºC.
- 1X Blocking Reagent: Prepare the appropriate volume for the number of samples being tested. IMMEDIATELY prior to using, dilute the provided 100X Blocking Reagent 1:100 in 1X PBS/0.1% BSA. Do not store.
- 1X Permeabilization Solution: Prepare the appropriate volume for the number of samples being tested. Prior to using, dilute the provided 10X Permeabilization Solution 1:10 in 1X PBS. Store at 4ºC.

- 1X Detection Reagent: Prepare the appropriate volume for the number of samples being tested. IMMEDIATELY prior to using, dilute the provided 250X Detection Reagent 1:250 in 1X PBS/0.1% BSA. Do not store.

Assay Protocol: Adherent Phagocytes

Note: This kit is suitable for adherent phagocytes only. For suspension cells, please use one of the following assay kits:

- *CytoSelect™ 96-well Phagocytosis Assay Kit (E.coli Substrate) #CBA-222*
- *CytoSelect™ 96-well Phagocytosis Assay Kit (Red Blood Cell Substrate) #CBA-220*

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	200	400
Zymosan Suspension (µL/well)	10	20	40
Fix Solution (µL/well)	100	200	400
Permeabilzation Solution (µL/well)	100	200	400
Detection Buffer (µL/well)	50	100	200

Table 1: Dispensing Volumes of Different Plate Formats

I. Phagocytosis of Zymosan

1. Harvest and resuspend phagocytic cells in culture medium at 1 – 5 x 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 Zymosan suspension to each well. Mix well and immediately transfer the plate to a cell culture incubator for 15 minutes – 2 hours. Each sample including a negative control without Zymosan particles should be assayed in duplicate.
4. Remove the culture medium by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.
5. Gently add 200 µL of cold, serum-free medium (e.g. DMEM, RPMI) to each well. Promptly remove the cold media by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. Repeat twice more.

   Note: For loosely attached cells, complete culture media is preferred to maintain cell attachment

II. Remove and block external particles

Note: Perform steps with care, gently adding solutions as to not disrupt cell attachment

1. Add 100 µL of Fixation Solution to each well, incubating 5 minutes at room temperature.
2. Promptly remove the Fixation Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.
3. Wash twice with 1X PBS.
4. Add 100 µL of prediluted 1X Blocking Reagent to each well (see Preparation of Reagents Section). Incubate the plate for 60 minutes at room temperature on an orbital shaker.
5. Promptly remove the Blocking Reagent by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. Wash three times with 1X PBS.

III. Detection of internalized particles

Note: Perform steps with care, gently adding solutions as to not disrupt cell attachment

1. Remove the PBS wash and add 100 µL of prediluted 1X Permeabilization Solution (see Preparation of Reagents Section) to each well, incubate 5 minutes at room temperature.
2. Promptly remove the Permeabilization Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. Wash once with 1X PBS.
3. Add 100 µL of prediluted 1X Detection Reagent to each well (see Preparation of Reagents Section). Incubate the plate for 60 minutes at room temperature on an orbital shaker.
4. Promptly remove the Detection Reagent Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. Wash three times with 1X PBS.
5. Add 50 µL of Detection Buffer to each well. Incubate the plate for 10 minutes at room temperature on an orbital shaker.
6. Initiate the reaction by adding 100 µL of Substrate. Incubate for 5-20 minutes at 37ºC.
7. Stop the reaction by adding 50 µL of the Stop Solution and mix by placing the plate on an orbital plate shaker for 30 seconds.
8. Read the absorbance of each well at 405 nm.

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 405 nm filter. One should use the data below for reference only. This data should not be used to interpret actual results.

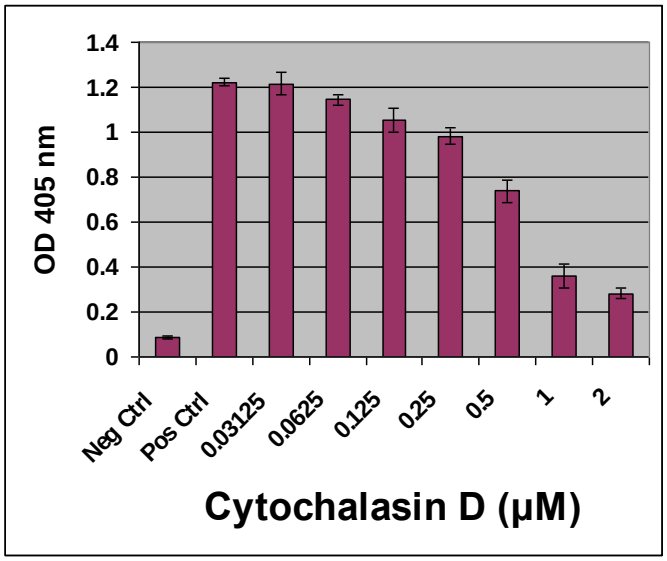


Figure 2. Inhibition of Raw 264.7 Macrophage Phagocytosis by Cytochalasin D. 50,000 cells/well of Raw 264.7 macrophages were seeded overnight in a 96-well plate. Cytochalasin D was used to pretreat Raw 264.7 cells for 1 hr at 37ºC before addition of Zymosan particles at 50:1 ratio. Phagocytosis was stopped after 30 minutes and the amount of engulfed Zymosan particles was determined as described in the Assay Protocol.

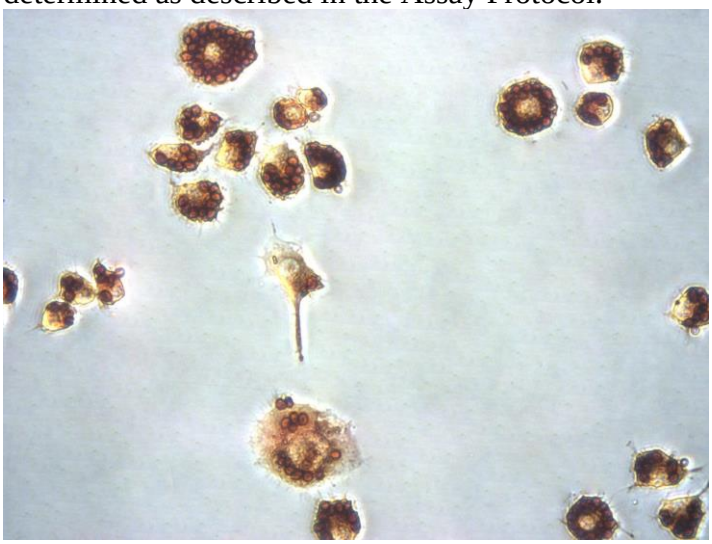


Figure 3. Zymosan Particles Engulfment by Raw 264.7 Macrophage.

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Recent Product Citations

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