Datasheet for ABIN2344909

CytoSelect™ 96-Well Phagocytosis Assay (Zymosan Substrate)

11 Publications

Go to Product page

Overview

Quantity:	96 tests
Reactivity:	Saccharomyces cerevisiae
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Analytical Method:	Quantitative
Characteristics:	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 2 accurately measure phagocytosis. The CytoSelect™ 96-well Phagocytosis Assay (Zymosan) provides a robust system for screening TLR ligands, phagocytosis activators or inhibitors. Each Trial Size kit provides sufficient quantities to perform 20 assays in a 96-well plate, 10 assays in a 48-well plate, or 5 assays in a 24-well plate.
Components:	1. Zymosan Suspension : One 0.25 mL tube of prelabeled Zymosan in PBS, 5 X 108 particles/mL 2. Fixation Solution : One 2 mL tube of 3.2% Buffered Formaldehyde Solution 3. Blocking Reagent (100X) : One 50 µL tube 4. 10X Permeabilization Solution : One 0.5 mL tube of PBS/1% Triton X-100 5. Detection Reagent (250X) : One 20 µL tube 6. Detection Buffer : One 2 mL tube 7. Substrate : Two 1.5 mL tubes 8. Stop Solution : Two 1.5 mL tubes

Product Details

	9. Phagocytosis Inhibitor : One 20 µL amber tube of 2 mM Cytochalasin D in DMSO
Material not included:	1. Phagocyte and Culture Medium 2. PBS, PBS/0.1 % BSA 3. 37 °C Incubator, 5 % CO2 Atmosphere 4. Light Microscope 5. 96-well Microtiter Plate Reader

Target Details

Background:	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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Fully quantify phagocytosis with no manual cell counting • High-throughput 96-well format • Convenient quantitation in a standard microplate reader
Reagent Preparation:	• Zymosan Suspension: Thaw Zymosan suspension at 4 °C. 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 37 °C, 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 4 °C.

- 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 4 °C. 4
- 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 Procedure:

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 100 200 400 Volume (µL/well) Zymosan Suspension 10 20 40 (µL/well) Fix Solution (µL/well) 100 200 400 Permeabilzation Solution 100 200 400 (µL/well) Detection Buffer 50 100 200 (µL/well) 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 105 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 37 °C, 5 % CO2.
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 5

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.

Application Details

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 37 °C.
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.

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store all kit components at 4°C.

Publications

Product cited in:

Rawat, Spector: "Development and characterization of a human microglia cell model of HIV-1 infection." in: **Journal of neurovirology**, Vol. 23, Issue 1, pp. 33-46, (2016) (PubMed).

Sapkota, Kharbanda, Wyatt: "Malondialdehyde-Acetaldehyde-Adducted Surfactant Protein Alters Macrophage Functions Through Scavenger Receptor A." in: **Alcoholism, clinical and experimental research**, Vol. 40, Issue 12, pp. 2563-2572, (2016) (PubMed).

Fiorcari, Martinelli, Bulgarelli, Audrito, Zucchini, Colaci, Potenza, Narni, Luppi, Deaglio, Marasca, Maffei: "Lenalidomide interferes with tumor-promoting properties of nurse-like cells in chronic lymphocytic leukemia." in: **Haematologica**, Vol. 100, Issue 2, pp. 253-62, (2015) (PubMed).

Liao, You, Li, Chang, Chang, Chen: "Cyclic GMP-dependent protein kinase II is necessary for macrophage M1 polarization and phagocytosis via toll-like receptor 2." in: **Journal of molecular medicine (Berlin, Germany)**, Vol. 93, Issue 5, pp. 523-33, (2015) (PubMed).

Zhang, Xue, Shah, Bermingham, Hinkle, Li, Rodrigues, Tabita-Martinez, Millar, Cuchel, Pashos, Liu, Yan, Yang, Gosai, VanDorn, Chou, Gregory, Morrisey, Li, Rader, Reilly: "Functional analysis and transcriptomic profiling of iPSC-derived macrophages and their application in modeling Mendelian disease." in: **Circulation research**, Vol. 117, Issue 1, pp. 17-28, (2015) (PubMed).

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