Datasheet for ABIN2344908

CytoSelect™ 96-Well Phagocytosis Assay (E. coli, Colorimetric Format)

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Overview

Quantity:	96 tests
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	CytoSelect™ 96-well Phagocytosis Assay (E. coli) uses enzyme-labeled, E. coli particles as a phagocytosis pathogen, however, it does not involve subjective manual counting of E. coli particles inside cells. Instead external particles are blocked before the colorimetric detection of engulfed E. coli particles (Figure 2). This format provides a quantitative, high-throughput method to accurately measure phagocytosis. The CytoSelect™ 96-well Phagocytosis Assay (E. coli) 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.
Components:	1. E. coli Suspension : One 1 mL tube of enzyme-labeled, fixative-inactivated E. coli in PBS/1% BSA 2. Fixation Solution : One 20 mL bottle of 3.2% Buffered Formaldehyde Solution 3. 10X Blocking Reagent : One 1.5 mL tube 4. 10X Permeabilization Solution : One 1.5 mL tube of PBS/1% Triton X-100 5. Substrate : One 12 mL amber bottle 6. Stop Solution : One 12 mL bottle 7. Phagocytosis Inhibitor : One 20 µL amber tube of 2 mM Cytochalasin D in DMSO

Product Details

Material not included:	1. Phagocytes 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.

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:	• E. coli Suspension: Thaw E. coli Suspension at 4 °C. Either nonopsonized or opsonized E. coli particles can be used in phagocytosis assay. For opsonization, it is recommended to use the entire 1 mL suspension (due to the small particle pellet during centrifugation). To opsonize E. coli 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 10X Blocking Reagent 1:10 in 1X PBS. Store at 4 °C. • 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

Assay Procedure:

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 100 200 400 (µL/well) E. coli Suspension 10 20 40 (µL/well) Fixation Solution 100 200 400 (µL/well) Permeabilization Solution 100 200 400 (µL/well) Table 1: Dispensing Volumes of Different Plate Formats

I. Phagocytosis of E. coli

1. Harvest and resuspend phagocytic cells in culture medium at 0.2 - 1 x 106 cells/mL or the appropriate concentration that yields >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 E. coli Suspension to each well. Mix well and immediately transfer the plate to a cell culture incubator for 3 - 6 hours, undisturbed. Each sample including a negative control without E. coli particles should be assayed in duplicate. Note: The E. coli particle is much smaller than other phagocytosis particles (e.g. Zymosan, RBC, latex bead), requiring longer incubation times to settle on phagocytic cells. Leave the plate undisturbed during this step.
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 four times total. Note: For loosely attached cells, complete culture media is preferred to maintain cell attachment

II. Removal and blocking of 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. 5
4. Add 100 µL of prediluted 1X Blocking Reagent to each well (see Preparation of Reagents Section). Incubate the plate for 30 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 twice with 1X PBS.
3. Promptly remove the PBS by gently aspirating or inverting the plate and blotting on a paper

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towel. Gently tap several times.

4. Initiate the reaction by adding 100 µL of Substrate. Incubate for 10-30 minutes at room temperature.
5. Stop the reaction by adding 100 µL of the Stop Solution and mix by placing the plate on an orbital plate shaker for 30 seconds.
6. Read the absorbance of each well at 450 nm. Assay Protocol: Suspension Phagocytes
    1. Harvest and resuspend phagocytic cells in culture medium at 0.2 - 1 x 106 cells/mL. Seed 100 µL in each well of a 96-well plate.
    2. Treat phagocytes with desired activators or inhibitors.
    3. Add 10 µL of E. coli Suspension to each well. Mix well and immediately transfer the plate to a cell culture incubator for 3 - 6 hours, undisturbed. Each sample including a negative control without E. coli particles should be assayed in duplicate. Note: The E. coli particle is much smaller than other phagocytosis particles (e.g. Zymosan, RBC, latex bead), requiring longer incubation times to settle on phagocytic cells. Leave the plate undisturbed during this step.
    4. Remove the culture medium by centrifugation and gentle aspiration.
    5. Add 200 µL of cold 1X PBS to each well. Promptly remove the PBS Solution by centrifugation and gentle aspiration.
    6. Add 100 µL of Fixation Solution to each well, incubate 5 minutes at room temperature.
    7. Promptly remove the Fixation Solution by centrifugation and gentle aspiration.
    8. Wash twice with 1X PBS.
    9. Add 100 µL of prediluted 1X Blocking Solution to each well (see Preparation of Reagents Section). Incubate the plate for 30 minutes at room temperature on an orbital shaker. 6
    10. Promptly remove the Blocking Solution by centrifugation and gentle aspiration. Wash three times with 1X PBS.
    11. Add 100 µL of prediluted 1X Permeabilization Solution (see Preparation of Reagents Section) to each well, incubate 5 minutes at room temperature.
    12. Promptly remove the Permeabilization Solution by centrifugation and gentle aspiration. Wash twice with 1X PBS.
    13. Promptly remove the PBS by centrifugation and gentle aspiration.
    14. Initiate the reaction by adding 100 µL of Substrate. Incubate for 10-30 minutes at room temperature.
    15. Stop the reaction by adding 100 µL of the Stop Solution and mix by placing the plate on an orbital plate shaker for 30 seconds.
    16. Read the absorbance of each well at 450 nm.

Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C
Storage Comment:	Upon receipt, aliquot and store the E. coli Suspension at -20°C to avoid multiple freeze/thaw

Handling

cycles. Store all other kit components at 4°C.

Publications

Product cited in:

García-Pascual, Martínez, Calvo, Ferrero, Villanueva, Pozuelo-Rubio, Soengas, Tormo, Simón, Pellicer, Gómez: "Evaluation of the potential therapeutic effects of a double-stranded RNA mimic complexed with polycations in an experimental mouse model of endometriosis." in: **Fertility and sterility**, Vol. 104, Issue 5, pp. 1310-8, (2015) (PubMed).

Gibson, Munns, Freytag, Barton, Veenstra, Bettahi, Bissonette, Wei: "Immunotherapeutic intervention with oncolytic adenovirus in mouse mammary tumors." in: **Oncoimmunology**, Vol. 4, Issue 1, pp. e984523, (2015) (PubMed).

Lakshmanan, Zhang, Nweze, Du, Harbrecht: "Glycogen synthase kinase 3 regulates IL-1? mediated iNOS expression in hepatocytes by down-regulating c-Jun." in: **Journal of cellular biochemistry**, Vol. 116, Issue 1, pp. 133-41, (2014) (PubMed).

Oh, Kang, Ooi, Choi, Sage, Rhee: "Overexpression of SPARC in human trabecular meshwork increases intraocular pressure and alters extracellular matrix." in: **Investigative ophthalmology & visual science**, Vol. 54, Issue 5, pp. 3309-19, (2013) (PubMed).

Muruganandan, Parlee, Rourke, Ernst, Goralski, Sinal: "Chemerin, a novel peroxisome proliferator-activated receptor gamma (PPARgamma) target gene that promotes mesenchymal stem cell adipogenesis." in: **The Journal of biological chemistry**, Vol. 286, Issue 27, pp. 23982-95, (2011) (PubMed).