Datasheet for ABIN2344907

CytoSelect™ 96-well Phagocytosis Assay (Red Blood Cell)

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Overview

Quantity:	96 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Analytical Method:	Quantitative
Characteristics:	CytoSelect™ 96-well Phagocytosis Assay does not involve subjective manual counting of erythrocytes. Instead cells are lysed and detected by the proprietary erythrocyte substrate in a microtiter plate reader (Figure 2). This format provides a quantitative, high-throughput method to 2 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.
Components:	1. Opsonization Solution : One 20 µL tube of Rabbit Anti-Sheep RBC, IgG 2. 10X Wash Solution : One 10 mL bottle 3. Lysis Buffer A : One 20 mL bottle 4. Lysis Buffer B : One 150 µL tube 5. 100X Substrate Solution : One 150 µL amber tube 6. Phagocytosis Inhibitor : One 20 µL amber tube of 2 mM Cytochalasin D in DMSO
Material not included:	1. Live sheep erythrocytes (MP Biomedicals or Lampire Biological Laboratories) 2. Phagocytes and Culture Medium 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. 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.

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:	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. 8Sheep Erythrocyte Opsonization: Resuspend live sheep erythrocytes at 1-5 x 10 cells/mL in sterile PBS. Add the Opsonization Solution to the sheep erythrocyte suspension at a 1:500 dilution. Mix 8 well and incubate at 37 °C for 30 minutes. Wash twice with sterile PBS and resuspend at 1-5 x 10 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. 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 100 300 500 Volume (µL/well) IgG Opsonized RBC 10 30 50 Suspension (µ L/well) 1X Wash Solution 200 600 1000 (µL/well) 1X PBS (µL/well) 200 600 1000 1X Lysis Buffer 120 240 360 (µL/well) Diluted Substrate 10 20 30 Solution (µL/well) Table 1: Dispensing Volumes of Different Plate Formats - • Harvest and resuspend phagocytic cells in culture medium at 1 5 x 10 cells/mL or the appropriate concentration that yields 50-80 % confluency after overnight incubation. Seed 100 µ L in each well of a 96-well plate and incubate overnight at 37 °C, 5 % CO2. • Treat phagocytes with desired activators or inhibitors. • 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. • Remove the culture medium by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. • Add 200 µL of cold 1X Wash Solution to each well. Incubate wells for 30 seconds on an orbital shaker. • Promptly remove the Wash Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. • Wash once with 200 µL of cold 1X PBS. • Promptly remove the PBS solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. • Add 120 µL of 1X Lysis Buffer to each well. Pipette each well 5-10 times to mix thoroughly. • Transfer 90 µL of the mixture to a 96-well microtiter plate. • 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. • Measure the absorbance at 610-630 nm in a 96-well microtiter plate reader. 5 Assay Protocol: Suspension Phagocytes 1. 6Harvest and resuspend phagocytic cells in culture medium at 0.2 - 1.0 x 10 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

Application Details

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)
13. Harvest and resuspend live erythrocytes in sterile PBS at 1-2 x 10 cells/mL. 7
14. Dilute erythrocytes 10-fold with 1X Lysis Buffer to 1-2 x 10 cells/mL.
15. Prepare a 2-fold, serial dilution in 1X Lysis Buffer, including a Lysis Buffer blank.
16. Transfer 90 µL of each dilution to a 96-well microtiter plate.
17. 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.
18. Measure the absorbance at 610-630 nm in a 96-well microtiter plate reader. 6

Restrictions:	For Research Use only

Handling

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

Publications

Product cited in:

Coffey, Rani, Betz, Pak, Haberstock-Debic, Pandey, Hollenbach, Gretler, Mant, Jurcevic, Sinha: " PRT062607 Achieves Complete Inhibition of the Spleen Tyrosine Kinase at Tolerated Exposures Following Oral Dosing in Healthy Volunteers." in: **Journal of clinical pharmacology**, Vol. 57, Issue 2, pp. 194-210, (2016) (PubMed).

Min, Cha, Kang: "Effects of waterborne nickel on the physiological and immunological parameters of the Pacific abalone Haliotis discus hannai during thermal stress." in: **Environmental science and pollution research international**, Vol. 22, Issue 17, pp. 13546-55, (2015) (PubMed).

Park, Kim, Kim, Lee, Kim, Shin, Yi: "Immunostimulatory effect of fermented red ginseng in the mouse model." in: **Preventive nutrition and food science**, Vol. 19, Issue 1, pp. 10-8, (2014) (PubMed).

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Aflaki, Stubblefield, Maniwang, Lopez, Moaven, Goldin, Marugan, Patnaik, Dutra, Southall, Zheng, Tayebi, Sidransky: "Macrophage models of Gaucher disease for evaluating disease pathogenesis and candidate drugs." in: **Science translational medicine**, Vol. 6, Issue 240, pp. 240ra73, (2014) (PubMed).

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