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Product Manual

# CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell, Colorimetric Format)

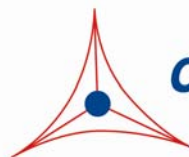
Catalog Number

CBA-220

96 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

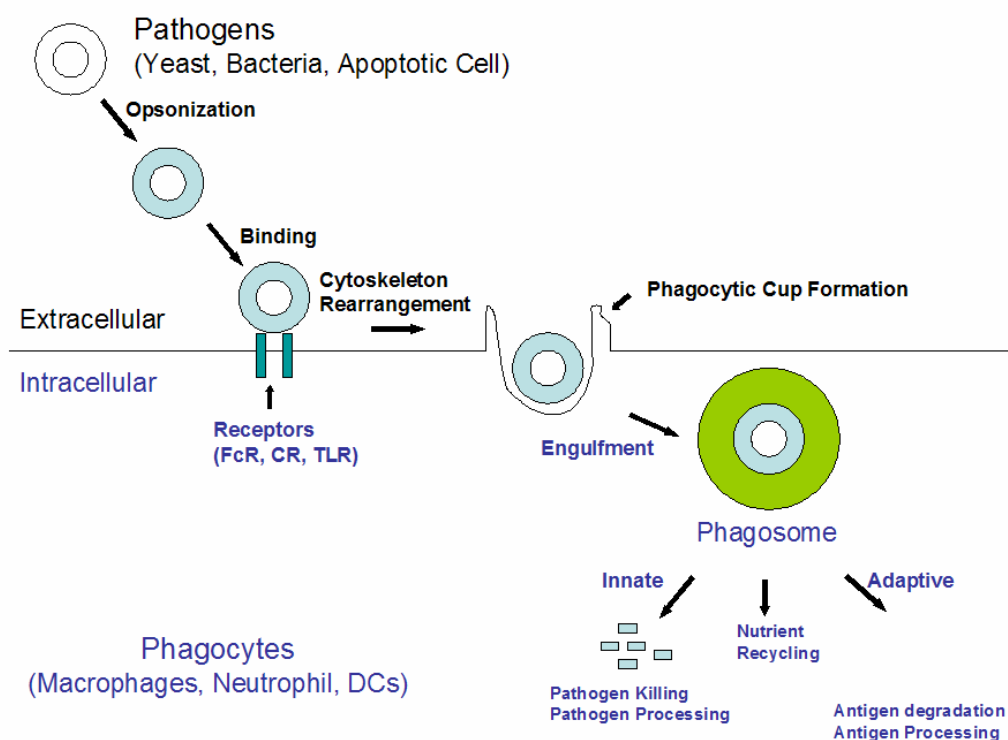
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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## 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.



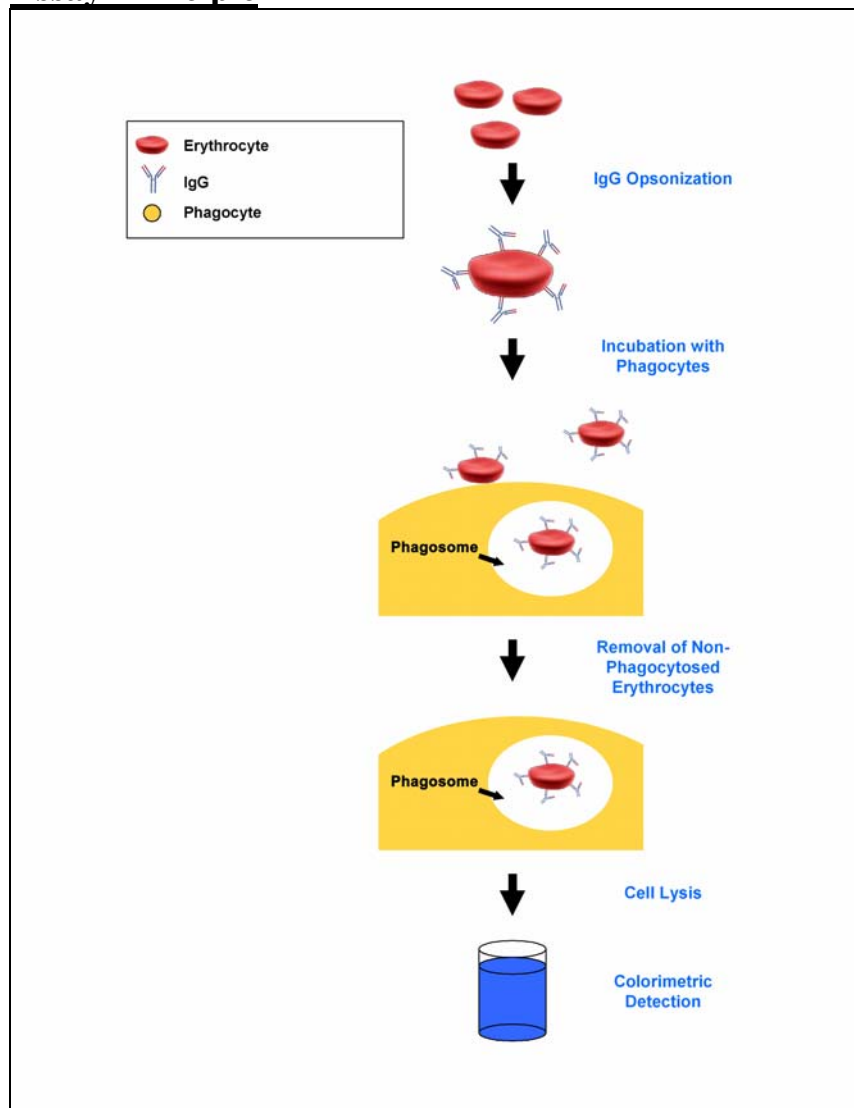
**Figure 1: Phagocytosis Processes.**

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.

Cell Biolabs' 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

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. CBA-224: CytoSelect™ 96-Well Phagocytosis Assay (Zymosan, 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. Opsonization Solution (Part No. 122001): One tube – 20  $\mu$ L of Rabbit Anti-Sheep RBC, IgG
2. 10X Wash Solution (Part No. 122002): One bottle – 10.0 mL
3. Lysis Buffer A (Part No. 122003): One bottle – 20.0 mL
4. Lysis Buffer B (Part No. 122004): One tube – 150  $\mu$ L
5. 100X Substrate Solution (Part No. 122005): One amber tube – 150  $\mu$ L
6. Phagocytosis Inhibitor (Part No. 122006): One amber tube – 20  $\mu$ L of 2 mM Cytochalasin D in DMSO

## **Materials Not Supplied**

1. Erythrocytes such as sheep red blood cells from MP Biomedicals (Cat.# 55876)
2. Culture Medium
3. 37°C Incubator, 5% CO<sub>2</sub> Atmosphere
4. Light Microscope
5. 96-well Microtiter Plate Reader

## **Storage**

Store all kit components at 4°C.

## **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 sheep erythrocytes at  $1-5 \times 10^8$  cells/mL in sterile PBS. Add the Opsonization Solution to the sheep erythrocyte suspension at a 1:500 dilution. Mix well and incubate at 37 °C for 30 minutes. Wash twice with sterile PBS and resuspend at  $1-5 \times 10^8$  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  $\mu$ L of 100X Substrate Solution to 900  $\mu$ L 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.

<b>Culture Dish</b>	<b>96-well</b>	<b>48-well</b>	<b>24-well</b>
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 37°C, 5% CO<sub>2</sub>.
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 – 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  $0.2 - 1.0 \times 10^6$  cells/mL. Seed 100  $\mu$ L in each well of a 96-well plate.
2. Treat phagocytes with desired activators or inhibitors.
3. Add 50  $\mu$ L 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  $\mu$ L 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  $\mu$ L of cold 1X PBS.
8. Promptly remove the PBS by centrifugation and gentle aspiration.
9. Add 120  $\mu$ L of 1X Lysis Buffer to each well. Pipette each well 5-10 times to mix thoroughly.
10. Transfer 90  $\mu$ L of the mixture to a 96-well microtiter plate.
11. Add 10  $\mu$ L 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 erythrocytes in sterile PBS at  $1-2 \times 10^8$  cells/mL.
2. Dilute erythrocytes 10-fold with 1X Lysis Buffer to  $1-2 \times 10^7$  cells/mL.
3. Prepare a 2-fold, serial dilution in 1X Lysis Buffer, including a Lysis Buffer blank.
4. Transfer 90  $\mu$ L of each dilution to a 96-well microtiter plate.
5. Add 10  $\mu$ L 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.

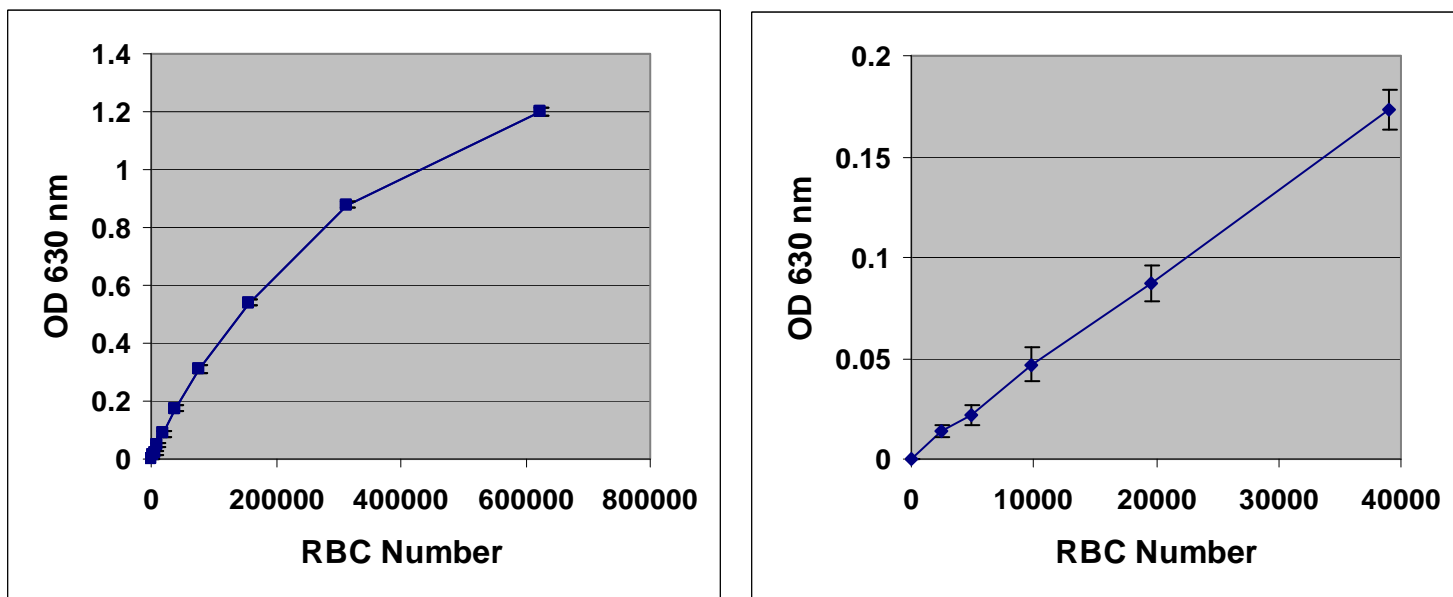
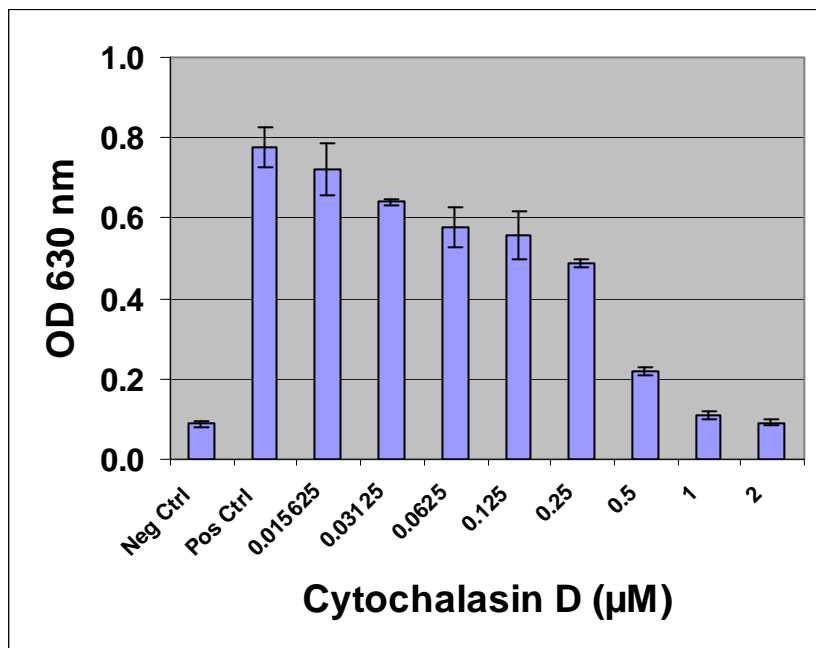
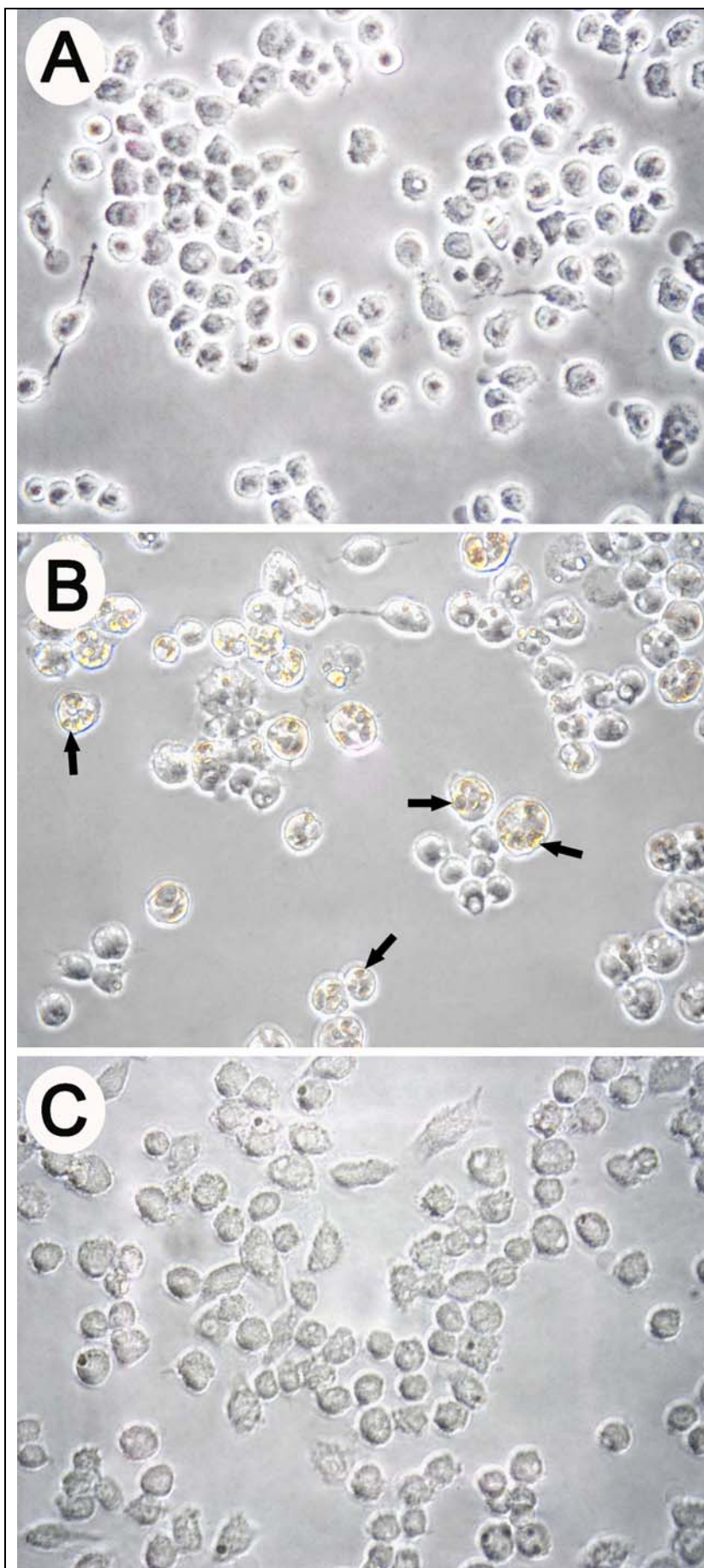


Figure 2. Sheep Red Blood Cell Dose Curve







**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 37°C 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. Top bar graph: Cytochalasin D dose response; 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).



## **References**

1. Sansonetti, P. (2001) *Semin. Immunol.* **13**:381–390.
2. Jutras I and Desjardins M. (2005) *Annu Rev Cell Dev Biol.* **21**:511-27.
3. Janeway, C. A., Jr., and Medzhitov R. (2002) *Annu. Rev. Immunol.* **20**:197–216.
4. Gordon, S. (2002) *Cell.* **111**:927–930.

## **Recent Product Citations**

1. Hamilton, C.M. et al. (2009). Fasciola hepatica tegumental antigen suppresses dendritic cell maturation and function. *Infect. Immun.* **77**:2488-2498.
2. Dowling, D.J. et al. (2010). Major secretory antigens of the helminth Fasciola hepatica activate a suppressive dendritic cell phenotype that attenuates Th17 cells but fails to activate Th2 immune responses. *Infect. Immun.* **78**:793-801.

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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