

---

Product Manual

# CytoSelect™ 96-Well Phagocytosis Assay (Zymosan Substrate)

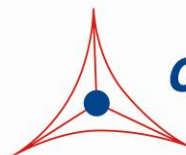
Catalog Number

CBA-224

96 assays

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

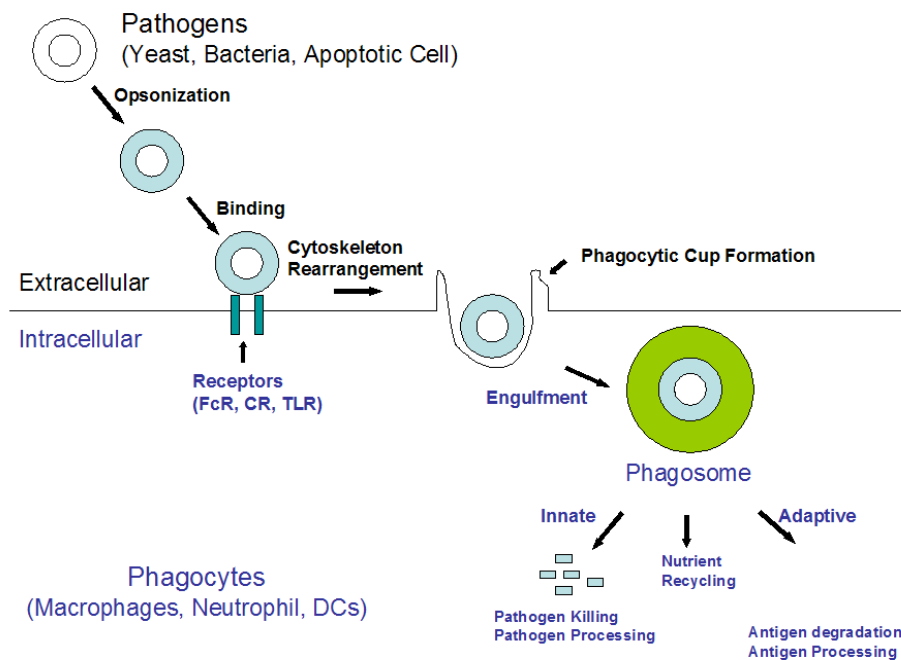
---



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

## Introduction

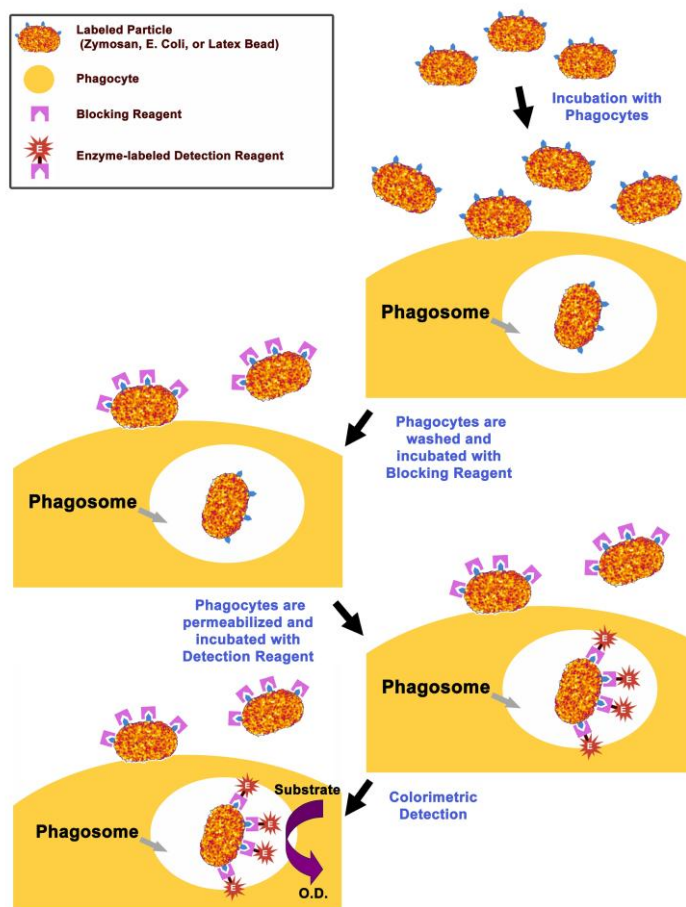
In mammals, phagocytosis by phagocytes (e.g., macrophages, dendritic cells, and neutrophils) is essential for a variety of biological events, including tissue remodeling and the continuous clearance of dying cells. Furthermore, phagocytosis represents an early and crucial event in triggering host defenses 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.**

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 pre-labeled 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.



## **Related Products**

1. CBA-222: CytoSelect™ 96-Well Phagocytosis Assay, E. coli
2. CBA-210: CytoSelect™ Leukocyte-Endothelium Adhesion Assay
3. CBA-212: CytoSelect™ Leukocyte Transmigration Assay

## **Kit Components (shipped at room temperature)**

1. Zymosan Suspension (Part No. 122401): One 1 mL tube of prelabeled Zymosan in PBS,  $5 \times 10^8$  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  $\mu$ L 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  $\mu$ L 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  $\mu$ L of 2 mM Cytochalasin D in DMSO

## **Materials Not Supplied**

1. Adherent Phagocytes and Culture Medium
2. PBS, PBS/0.1% BSA
3. 37°C Incubator, 5% CO<sub>2</sub> Atmosphere
4. Light Microscope
5. 96-well Microtiter Plate
6. 96-well Microtiter Plate Reader

## **Storage**

Store all kit components at 4°C.

## **Preparation of Reagents**

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

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 ( $\mu\text{L}/\text{well}$ )	100	200	400
Zymosan Suspension ( $\mu\text{L}/\text{well}$ )	10	20	40
Fix Solution ( $\mu\text{L}/\text{well}$ )	100	200	400
Permeabilization Solution ( $\mu\text{L}/\text{well}$ )	100	200	400
Detection Buffer ( $\mu\text{L}/\text{well}$ )	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  $1 - 5 \times 10^5$  cells/mL or the appropriate concentration that yields 50-80% confluency after overnight incubation. Seed 100  $\mu\text{L}$  in each well of a 96-well plate and incubate overnight at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$ .
2. Treat phagocytes with desired activators or inhibitors.
3. Add 10  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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

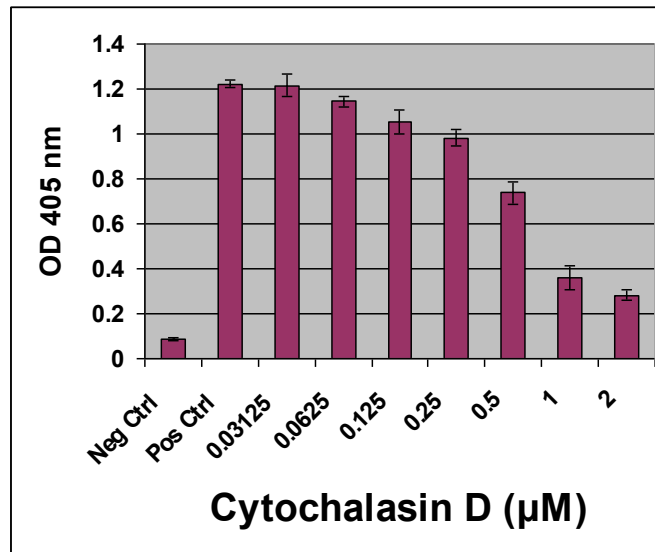
*Note: Perform steps with care, gently adding solutions as to not disrupt cell attachment*

1. Remove the PBS wash and add 100  $\mu\text{L}$  of prediluted 1X Permeabilization Solution (see Preparation of Reagents Section) to each well, incubate 5 minutes at room temperature.

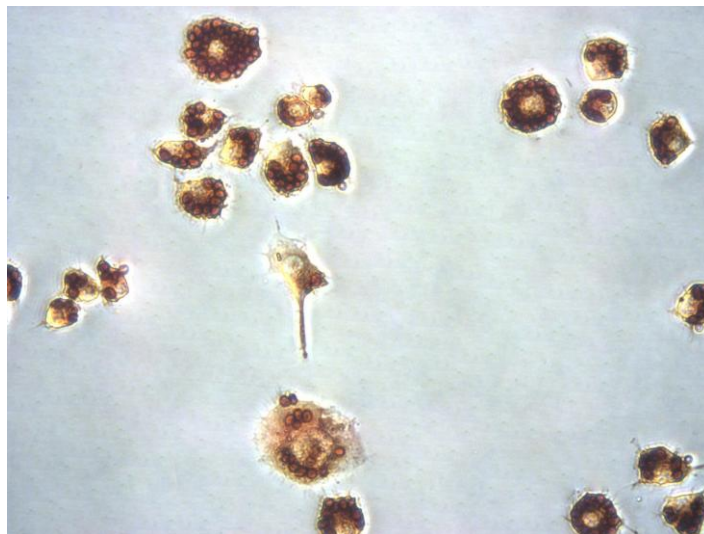
2. Promptly remove the Permeabilization Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times. Wash once with 1X PBS.
3. Add 100  $\mu$ L of prediluted 1X Detection Reagent to each well (see Preparation of Reagents Section). Incubate the plate for 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  $\mu$ L 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  $\mu$ L of Substrate. Incubate for 5-20 minutes at 37°C.
7. Stop the reaction by adding 50  $\mu$ L 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. 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 37°C 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.**

### **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. Zhang, Y. et al. (2025). Prostate cancer cell-derived exosomes inhibit macrophage phagocytosis through EIF3B-mediated exosomal sorting of miR-100-5p. *Sci Rep.* **15**(1):26138. doi: 10.1038/s41598-025-11799-w.
2. Chandra, N. et al. (2024). Evaluation of immunity enhancing potential of Ocimum sanctum (TulsiOdaat™) through an in-vitro cytotoxicity and phagocytic activity assessment on mouse macrophage RAW 264.7 cell line. *Int J Herb Med.* **12**(2):01-04. doi: 10.22271/flora.2024.v12.i2a.923.
3. Jang, H. et al. (2022). Specific histamine regulating activity of surface-modified yeast vacuoles by histamine- binding protein and its immune-enhancing effect. *Microb Biotechnol.* doi: 10.1111/1751-7915.14116.
4. Narayanaperumal, J. et al. (2022). A randomized double blinded placebo controlled clinical trial for the evaluation of green coffee extract on immune health in healthy adults. *J Tradit Complement Med.* doi: 10.1016/j.jtcme.2022.01.007.
5. Naveenchandra. et al. (2021). In-vitro cytotoxicity and phagocytic activity of AQUATURM®-water soluble extract of Curcuma longa, on mouse macrophage RAW 264.7 Cell line. *Int J Herb Med.* **9**(5):38-41.
6. Kim, S. et al. (2021). Immunostimulatory activity of stem bark of Kalopanax pictus in RAW 264.7 macrophage. *J Herb Med.* doi: 10.1016/j.hermed.2021.100504.
7. Park, E. et al. (2021). In Vivo Evaluation of Immune-Enhancing Activity of Red Gamju Fermented by Probiotic Levilactobacillus brevis KU15154 in Mice. *Foods.* **10**(2):253. doi: 10.3390/foods10020253.

8. Shin, J. et al. (2020). Immunomodulatory Effect of a *Salvia plebeia* R. Aqueous Extract in Forced Swimming Exercise-induced Mice. *Nutrients*. **12**(8):E2260. doi: 10.3390/nu12082260.
9. Vay, S.U. et al. (2020). The impact of hyperpolarization-activated cyclic nucleotide-gated (HCN) and voltage-gated potassium KCNQ/Kv7 channels on primary microglia function. *J Neuroinflammation*. **17**(1):100. doi: 10.1186/s12974-020-01779-4.
10. Zuo, P. et al. (2020). Protease-activated receptor 2 deficiency in hematopoietic lineage protects against myocardial infarction through attenuated inflammatory response and fibrosis. *Biochem Biophys Res Commun*. doi: 10.1016/j.bbrc.2020.03.077.
11. Trivedi, M.K. et al. (2020). Solid and liquid state characterization of tetrahydrocurcumin using XRPD, FT-IR, DSC, TGA, LC-MS, GC-MS, NMR and its biological activities. *J Pharm Anal*. doi: 10.1016/j.jpha.2020.02.005.
12. Dashdulam, D. et al. (2020). Osteopontin heptamer peptide containing the RGD motif enhances the phagocytic function of microglia. *Biochem Biophys Res Commun*. pii: S0006-291X(20)30175-3. doi: 10.1016/j.bbrc.2020.01.100.
13. Rabenstein, M. et al. (2020). Crosstalk between stressed brain cells: direct and indirect effects of ischemia and aglycemia on microglia. *J Neuroinflammation*. **17**(1):33. doi: 10.1186/s12974-020-1697-8.
14. Abumaree, M.H. et al. (2019). Decidua Basalis Mesenchymal Stem Cells Favor Inflammatory M1 Macrophage Differentiation In Vitro. *Cells*. **8**(2). pii: E173. doi: 10.3390/cells8020173.
15. Kim, S. et al. (2019). Immune-enhancing screening of fourteen plants on murine macrophage RAW 264.7 cells. *Trop J Pharm Res*. **18**(1): 86. doi: 10.4314/tjpr.v18i1.13.
16. Al-Kushi, A.G. et al. (2018). Antioxidant effect of royal jelly on immune status of hyperglycemic rats. *Phcog Mag*. **14**:528-33. doi: 10.4103/pm.pm\_87\_18.
17. Vay, S.U. et al. (2018). The plasticity of primary microglia and their multifaceted effects on endogenous neural stem cells in vitro and in vivo. *J Neuroinflammation*. **15**(1):226. doi: 10.1186/s12974-018-1261-y.
18. Trivedi, M.K. et al. (2017). Immunomodulatory potential of nanocurcumin-based formulation. *Inflammopharmacol*. **25** (6): 609-619.
19. Sapkota, M. et al. (2016). Malondialdehyde-acetaldehyde-adducted surfactant protein alters macrophage functions through Scavenger Receptor A. *Alcoholism Clin. Exp. Res*. **40**:2563-2572.
20. Rawat, P. & Spector, S. A. (2016). Development and characterization of a human microglia cell model of HIV-1 infection. *J Neurovirol*. doi:10.1007/s13365-016-0472-1.
21. Beringer, P. M. et al. (2015). Rhesus  $\theta$ -defensin-1 (RTD-1) exhibits in vitro and in vivo activity against cystic fibrosis strains of *Pseudomonas aeruginosa*. *J Antimicrob Chemother*. doi: 10.1093/jac/dkv301.
22. Lee, S. G. et al. (2015). Immunostimulatory polysaccharide isolated from the leaves of *Diospyros kaki* Thumb modulate macrophage via TLR2. *Int J Biol Macromol*. **79**:971-982.
23. Jung, J. Y. et al. (2015). *Lactobacillus sakei* K040706 evokes immunostimulatory effects on macrophages through TLR 2-mediated activation. *Int Immunopharmacol*. doi: 10.1016/j.intimp.2015.05.037.
24. Zhang, H. et al. (2015). Functional analysis and transcriptomic profiling of iPSC-derived macrophages and their application in modeling mendelian disease. *Circ Res*. doi:10.1161/CIRCRESAHA.117.305860.
25. Fiorcari, S. et al. (2015). Lenalidomide interferes with tumor-promoting properties of nurse-like cells in chronic lymphocytic leukemia. *Haematologica*. **100**:253-262.

26. Liao, W. T. et al. (2014). Cyclic GMP-dependent protein kinase II is necessary for macrophage M1 polarization and phagocytosis via toll-like receptor 2. *J Mol Med (Berl)*. doi: 10.1007/s00109-014-1236-0.
27. Kasat, K. et al. (2014). Anti-inflammatory actions of endogenous and exogenous interleukin-10 versus glucocorticoids on macrophage functions of the newly born. *J Perinatol*. **34**:380-385.
28. Haselow, K. et al. (2013). Bile acids PKA-dependently induce a switch of the IL-10/IL-12 ratio and reduce proinflammatory capability of human macrophages. *J. Leukoc. Biol*. **94**:1253-1264.
29. Pierce, L.M. et al. (2012). Effect of heavy metal tungsten alloy particles on oxidative product formation and phagocytosis by lung macrophages. *Am. J. Respir. Crit. Care Med*. **185**:A4666.
30. Polancec, D.S. et al. (2012). Azithromycin drives in vitro GM-CSF/IL-4-induced differentiation of human blood monocytes toward dendritic-like cells with regulatory properties. *J Leukoc Biol*. **91**:229-243.

## **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.

## **Contact Information**

Cell Biolabs, Inc.  
5628 Copley Drive  
San Diego, CA 92111  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2007-2025: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.