Version 3e Last updated 29 June 2020

# ab211156 Phagocytosis Assay – Zymosan Substrate

For the quantitative and accurate measurement of phagocytosis using Zymosan particles as phagocytosis pathogen.

View kit datasheet: www.abcam.com/ab211156
(use www.abcam.cn/ab211156 for China, or www.abcam.co.jp/ab211156 for Japan)

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

# 1. Overview

Phagocytosis Assay – Zymosan substrate (ab211156) provides a robust system for screening TLR ligands, phagocytosis activators or inhibitors. The assay uses pre-labeled Zymosan particles as a pathogen for triggering phagocytosis. The engulfed particles react with a specific substrate to produce a colorimetric signal that can be detected at OD 405 nm. External particles are blocked prior the reaction with a blocking reagent, ensuring the signal is directly proportional to the amount of internalized particles.

This format provides a quantitative, high-throughput method to accurately measure phagocytosis, and avoids subjective manual counting of Zymosan particles inside cells.

Each 20 tests kit provides sufficient quantities to perform 20, 10 or 5 tests in a 96-,48- or 24-well plate respectively.

Each 96 tests kit provides sufficient quantities to perform 96, 48 or 24 tests in a 96-, 48- or 24-well plate respectively.

Each 5 x 96 tests kit provides sufficient quantities to perform 5 x 96, 48 or 24 tests in a 96-, 48- or 24-well plate respectively.

In mammals, phagocytosis by phagocytes (i.e., macrophages, dendritic cells and neutrophils) is essential for a variety of biological events such as 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. 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.

Zymosan (*Saccharomyces cerevisiae*) is prepared from yeast cell wall and consists of protein-carbohydrate complexes, and it is a commonly used pathogen in phagocytosis assays.

## 2. Protocol Summary

Incubate pre-labeled Zymosan particles with phagocytes

Wash phagocytes

Incubate phagocytes with Blocking Reagent

Permeabilize phagocytes and incubate with Substrate

Measure absorbance at OD 405 nm

## 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 4. Storage and Stability

**Store kit at 4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

**Δ Note**: Avoid multiple freeze/thaw cycles.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item | Quantity | | | Storage Condition (Before/After prep) |
|---|---|---|---|---|
| | 20 tests | 96 tests | 5 x 96 tests | |
| 10X Permeabilization Solution | 500 µL | 1.5 mL | 1.5 mL | 4°C |
| 250X Detection Reagent | 20 µL | 50 µL | 50 µL | 4°C |
| Blocking Reagent (100x) | 50 µL | 200 µL | 200 µL | 4°C |
| Detection Buffer | 2 mL | 10 mL | 10 mL | 4°C |
| Fixation Solution | 2 mL | 20 mL | 20 mL | 4°C |
| 2mM Phagocytosis Inhibitor | 20 µL | 20 µL | 20 µL | 4°C |
| Stop Solution | 2 x 1.5 mL | 12 mL | 12 mL | 4°C |
| Substrate | 2 x 1.5 mL | 12 mL | 12 mL | 4°C |
| Zymosan suspension | 250 µL | 1 mL | 1 mL | 4°C |

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Phagocytes (RAW 264.7 macrophages were used in this assay, but any phagocyte can be used)
- Phagocytes activators or inhibitors, as required
- Culture medium, appropriate for phagocytes
- 37°C Incubator, 5% $CO_2$ atmosphere
- Microplate reader capable of measuring absorbance at OD 405 nm
- Sterile 96 well plate/ 48 well plate/ 24 well plate with clear flat bottom
- MilliQ water or other type of double distilled water (dd$H_2O$)
- Sterile PBS
- Sterile PBS/0.1% BSA solution
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Orbital shaker

For opsonization step (optional):

- Serum or IgG solution (from same species as macrophages)

## 8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

# 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 9.1 10X Permeabilization Solution (PBS/1% Triton X-100):

Dilute the provided 10X Permeabilization Solution 1:10 in PBS to prepare **1X Permeabilization Solution**. Prepare the appropriate volume for the number of samples being tested. Store at 4°C.

### 9.2 250X Detection Reagent:

Immediately prior using, dilute the provided 250X Detection Reagent 1:250 in PBS/0.1% BSA to prepare **1X Detection Reagent**. Prepare the appropriate volume for the number of samples being tested. Do not store diluted Detection Reagent.

Unused 250X Detection Reagent can be stored at 4°C.

### 9.3 Blocking Reagent (100X):

Immediately prior to using, dilute the provided 100X Blocking Reagent 1:100 in PBS/0.1% BSA to prepare **1X Blocking Reagent**. Prepare the appropriate volume for the number of samples being tested. Do not store diluted Blocking Reagent.

Unused Blocking reagent (100X) can be stored at 4°C.

### 9.4 Detection Buffer:

Ready to use as supplied. Store at 4°C.

### 9.5 Fixation Solution (3.2% buffered formaldehyde solution):

Ready to use as supplied. Store at 4°C.

### 9.6 Phagocytosis Inhibitor (cytochalasin D in DMSO):

Ready to use as supplied. Store at 4°C.

### 9.7 Stop Solution:

Ready to use as supplied. Store at 4°C.

### 9.8 Substrate:

Ready to use as supplied. Store at 4°C.

### 9.9 Zymosan Suspension (5 x $10^8$ particles/mL):

Before using the Zymosan suspension for the first time, thaw suspension at 4°C. Zymosan particles are ready to use.

This assay can be performed with opsonized or non-opsonized Zymosan particles.

**For opsonization, it is recommended to use the entire suspension due to the small particle pellet during centrifugation.**

To opsonize Zymosan particles: incubate particles with desired serum or IgG for 30 minutes at 37°C, pellet particles by centrifugation and wash twice with sterile PBS. Prior to using, resuspend the opsonized particles in the same volume of sterile PBS. Store at 4°C.

## 10. Assay Procedure

- This kit is suitable for adherent phagocytes only.
- We recommend that you assay all controls and samples in duplicate.
- The following protocol is written for a 96-well plate format. Refer to the table below for the appropriate dispensing volume of other plate formats.

| Culture dish | 96-well | 48-well | 24-well |
|---|---|---|---|
| Phagocytose Seeding volume (µL/well) | 100 | 200 | 400 |
| E.coli Suspension (µL/well) | 10 | 20 | 40 |
| Fixation Solution (µL/well) | 100 | 200 | 400 |
| Permeabilization Solution (µL/well) | 100 | 200 | 400 |

### 10.1 Phagocytosis of Zymosan:

10.1.1 Harvest and resuspend phagocytic cells in culture medium at 1 – 5 x $10^5$ 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% $CO_2$.

10.1.2 Treat phagocytes with desired activators or inhibitors. Optional negative treated control: treat cells with the cytochalasin D provided (see Step 9.6)

10.1.3 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.

**Δ Note:** Prepare a negative control without Zymosan particles.

10.1.4 Remove the culture medium by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.

10.1.5 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.

**Δ Note:** For loosely attached cells, complete culture media is preferred to maintain cell attachment.

10.1.6 Wash with culture medium twice.

### 10.2 Removal and blocking of external particles:

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

10.2.1 Remove the culture medium and add 100 µL of Fixation Solution to each well. Incubate the plate for 5 minutes at room temperature.

10.2.2 Promptly remove the Fixation Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.

10.2.3 Wash twice with PBS.

10.2.4 Add 100 µL of 1X Blocking Reagent to each well (see Reagent Preparation section). Incubate the plate for 60 minutes at room temperature on an orbital shaker.

10.2.5 Promptly remove the Blocking Reagent by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.

10.2.6 Wash three times with 1X PBS

### 10.3 Detection of internalized particles:

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

10.3.1 Remove the PBS wash and add 100 µL of prediluted 1X Permeabilization Solution (see Step 9.1) to each well. Incubate the plate for 5 minutes at room temperature.

10.3.2 Promptly remove the 1X Permeabilization Solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.

10.3.3 Wash once with PBS.

10.3.4 Add 100 µL of prediluted 1X Detection Reagent (see Step 9.2) to each well. Incubate the plate for 60 minutes at room temperature on an orbital shaker at low speed.

10.3.5 Promptly remove the Detection Reagent solution by gently aspirating or inverting the plate and blotting on a paper towel. Gently tap several times.

10.3.6 Wash three times with PBS.

10.3.7 Add 50 µL of Detection Buffer to each well. Incubate the plate for 10 minutes at room temperature on an orbital shaker at low speed.

10.3.8 Initiate reaction by adding 100 µL of substrate. Incubate for 5 – 20 minutes at 37°C.

10.3.9 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.

10.3.10 Read absorbance at OD 40 5nm.

# 11. Typical data

Data provided for **demonstration purposes** only.

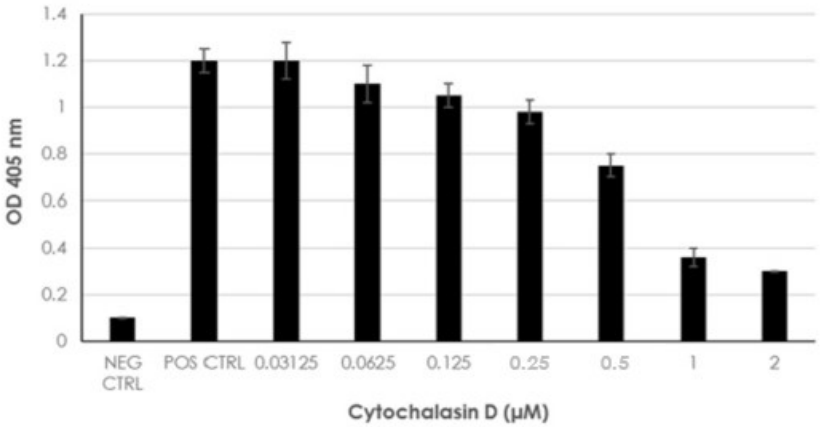


**Figure 1**. Inhibition of Raw 264.7 Macrophage phagocytosis by cytochalasin D. 5 x $10^4$ Raw 264.7 cells/well were seeded overnight in a 96-well plate. Cytochalasin D was used to pretreat Raw 264.7 cells for 1 hour at 37°C before addition of Zymosan particles at 50:1 ratio. Phagocytosis was stopped after 30 minutes and the amount of engulfed Zymosan particles was determined as described in the assay protocol.

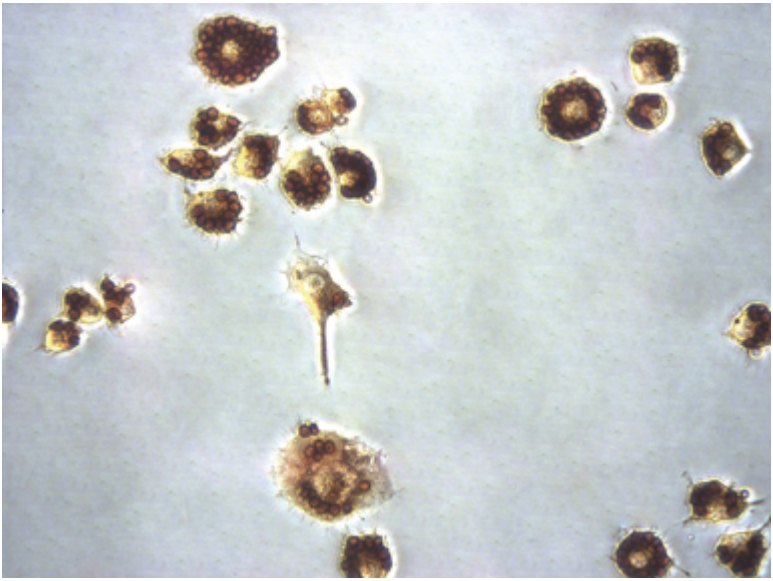

**Figure 1**. Zymosan particle engulfment by Raw 264.7 macrophages.

## 12. FAQs

### Q. What phagocytes are compatible with the assay?

A. We used Raw 264.7 macrophages, but any phagocyte can be used. You might need to optimize the cell number for your experimental setting though.

### Q. Which media should I use?

A. The medium will be specific for the phagocyte that you are using. Any phagocyte can be used in this assay with the media that is regularly used for culturing that phagocyte.

### Q. How can I represent/quantify the results?

A. This is a cell-based assay and therefore there are no standards for quantification. The results should be reported as relative comparison of ODs; for example: no particle control, phagocyte sample and phagocyte sample with treatment.

### Q. Does this assay require opsonization of the substrate?

A. Zymosan used in this assay is from yeast, a natural pathogen for phagocytes and therefore it does not require opsonization. However, if you prefer to opsonize Zysoman particles, the best method is to opsonize with serum from the same species as your phagocyte.

# 13. Notes

## Technical Support



### For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)