ELISA PRODUCT INFORMATION & MANUAL

Green Zymosan Phagocytosis Assay Kit (Fluorometric)

NBP2-54859

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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1. Overview

Green Zymosan Phagocytosis Assay Kit (Fluorometric) (NBP2-54859) utilizes pre- labeled Zymosan particles as a tool for rapid and accurate detection and quantification of *in vitro* phagocytosis by fluorescent microscope, spectrophotometer or flow cytometry. The kit provides a robust screening system for activators and/or inhibitors of phagocytosis and Toll-like receptors ligands (TLR).

Prepare cells.

Add effector and incubate for 1 hour at 37 °C, 5 % CO_2.

Add Zymosan slurry and incubate at 37 °C, 5 % CO_2 for 2 - 3 hours.

Harvest cells by centrifugation. Wash 3 times with ice cold Phagocytosis Assay Buffer containing effector. Suspend cells in ice cold Phagocytosis Assay Buffer.

Analyze by scanning or imaging of all experimental and control wells in the plate reader at Ex/Em at 490/520 nm, respectively.

2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)
Phagocytosis Assay Buffer	2 x 100 mL	4°C
Buffer Additive	2 x 1 mL	4°C
Green Zymosan	600 µL	4°C
10X Quenching Solution	500 µL	4°C

3. Materials Required, Not Supplied

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

- Multi-well spectrophotometer measuring excitation and emission at 490 and 520 nm, respectively.
- Fluorescent microscope (optional) for observation or flow cytometer equipped with laser capable of excitation at 488 nm.
- Stock solutions of effectors of interest (for example, cytochalasin D, inhibitor of actin cytoskeletal rearrangement)
- 6-, 12-, 24-, or 96-well opaque plates with clear bottoms for measurement of fluorescence.

  Δ Note: 6-, 12-, 24-, or 96-well clear plates should be used only for cell culturing. The measurement of fluorescence should be performed in opaque plates with clear bottoms. Alternatively, sterile opaque plates with clear bottoms can be used for both, culturing and measurements.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Phagocytosis Assay Buffer

Combine one entire vial of Buffer Additive with one Phagocytosis Assay Buffer, mix well. Use sterile pipetting technique throughout the assay.

5.2 Green Zymosan

Before each use, equilibrate the suspension to room temperature and vortex gently for 5 seconds.

5.3 Quenching Solution

Dilute the content of the vial into 4.5 mL of 1X Phagocytosis Assay Buffer.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Add 0, 1, 2, 3 and 4 µL of Green Zymosan slurry into a series of wells in 96-well plate.
2. Adjust the volume to 100 µL with Phagocytosis Assay Buffer. Mix well.
3. Immediately measure fluorescence using plate reader at Ex/Em 490/520 nm respectively.
4. Subtract 0 Standard reading from all the readings and plot the Standard Curve.

7. Sample Preparation

Preparation of control and experimental wells:

1. Subculture cells capable of phagocytosis (adherent or suspension) in appropriate medium.
2. The day prior to the experiment obtain a culture of 1 - 5 x 10^6 viable cells/mL.
3. Aliquot 100 µL of the cell culture per well omitting the negative control wells and incubate the plate overnight at 37 °C, 5 % CO_2.
4. Next day, change the media and proceed to the phagocytosis effector assay.
5. Your experiment should always consist of parallel negative, positive and experimental wells respectively.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Phagocytosis effector assay:

1. Add 100 µL of cell culture media containing your effector of interest (not provided in the kit) at desired concentration (e.g. 20 µM Cytochalasin D) to each of the experimental wells.
2. Aliquot 100 µL of media to each of the positive and 200 µL media to each of the negative control wells respectively.
3. Incubate for 1 hour at 37 °C, 5 % CO_2.

8.2 Phagocytosis of Green Zymosan:

1. Add 5 µL of Zymosan slurry to all the wells.
2. Immediately transfer the plate back to the incubator for 2 - 3 hours. The incubation time may be adjusted according to your protocol.

8.3 Sample preparation:

1. Harvest the cells by centrifugation for 5 minutes at 400 x *g*.
2. Carefully aspirate off the media and gently resuspend the cell pellets in 300 µL of ice cold Phagocytosis Assay Buffer containing the effector of interest at the same concentration as in the assay media.
3. Centrifuge for 5 minutes at 400 x *g* and repeat the washing step 3 more times.
4. Finally, suspend the cells in 200 µL of ice cold Phagocytosis Assay Buffer and proceed to the preferred method of detection.

8.4 Detection:

1. Cells can be analyzed by scanning or imaging of all experimental and control wells in the plate reader at Ex/Em at 490/520 nm, respectively.
2. Optional: For plate reader and microscope detection, re-suspend the cell pellets in 50 µL of the diluted Quenching

Solution and incubate for two minutes at room temperature. Centrifuge for 5 minutes at 400 x *g* and carefully remove the Quenching solution. Suspend the cells in 200 µL of ice cold Phagocytosis Assay Buffer.

3. For microscope and plate reader: Transfer 100 µL of each control and sample into a separate well and record the fluorescence.
4. For flow cytometry: Transfer 100 µL of cell suspension into a 900 µL of the Phagocytosis Assay Buffer in the flow cytometry compatible vessel. Analyze immediately in the FL1 channel of flow cytometer equipped with laser capable of excitation at 488 nm.

9. Data Analysis

To calculate the net phagocytosis subtract the average RFU of the no-cell negative-control wells from all positive control and experimental wells. The phagocytosis response to the experimental effector (% Effect) can be expressed as follows:

$$\% \text{ Effect} = \frac{\text{Net experimental phagocytosis } \times 100\%}{\text{Net positive control phagocytosis}}$$

10. Typical Data

Data provided **for demonstration purposes only**.

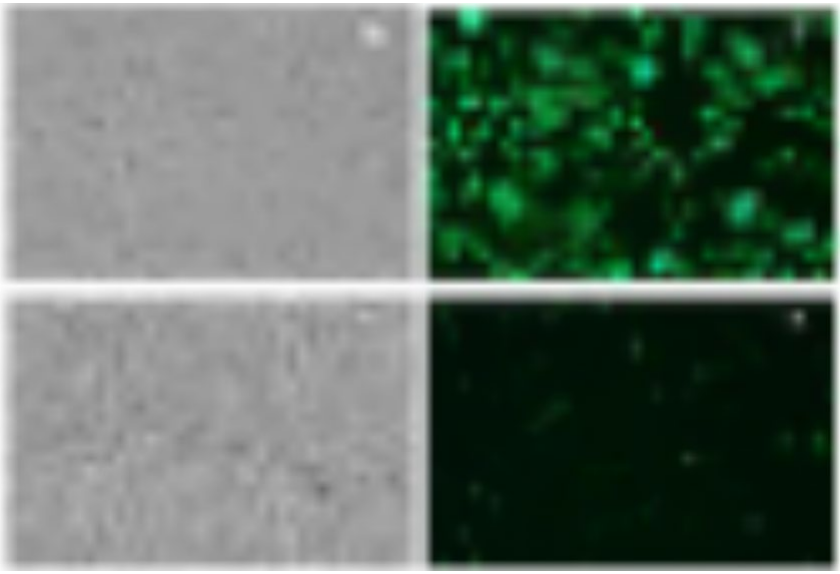

Figure 1. J774 macrophages were seeded overnight at 5 x 10^5 of viable cells/well. The next day the cells were pretreated with 20 µM Cytochalasin D for 1 h at 37°C prior to addition of 5 µL of Zymosan particles. Phagocytosis was conducted for 2 hours and the amount of engulfed Zymosan was determined as described in the Assay Protocol. Inhibition of phagocytosis. Panel A and B: images of non-treated cells. Panel C and D: treatment with Cytochalasin D.

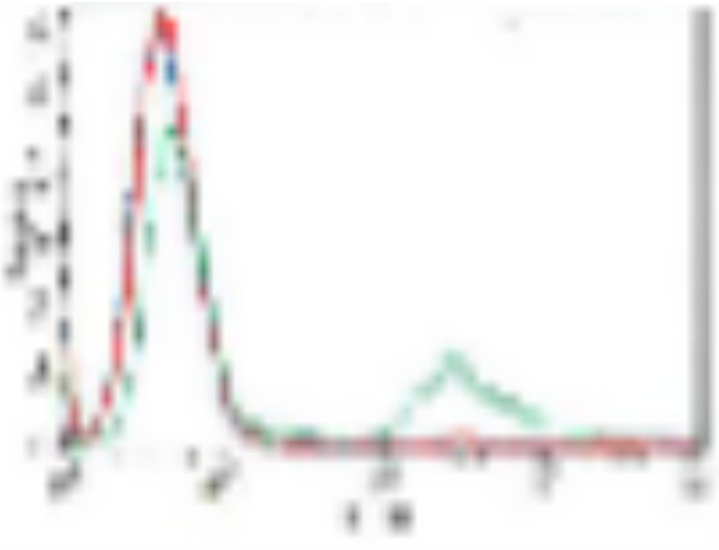

Figure 2. J774 macrophages were seeded overnight at 5 x 10^5 of viable cells/well. The next day the cells were pretreated with 20 µM Cytochalasin D for 1 h at 37°C prior to addition of 5 µL of Zymosan particles. Phagocytosis was conducted for 2 hours and the amount of engulfed Zymosan was determined as described in the Assay Protocol. Flow cytometry plot. Red line: untreated control cells; green line: macrophages with engulfed Zymosan particles; blue line: inhibition of phagocytosis by Cytochalasin D.

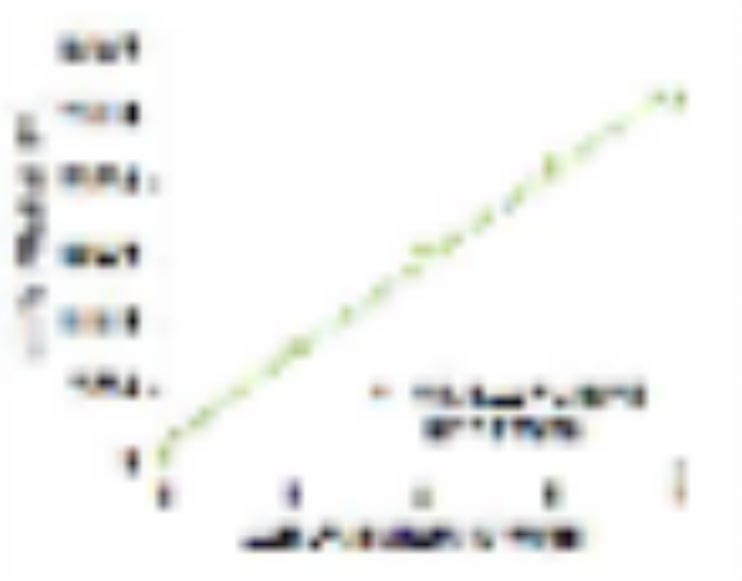

Figure 3. Zymosan Standard curve.

11. Notes