Phagocytosis Assay Kit (Green Zymosan)

PromoKine

For rapid and accurate detection and quantification of in vitro phagocytosis

Instruction Manual

Catalog Number	PK-CA577-K397
Description	Phagocytosis in mammals serves as an important first line defense mechanism against invading pathogens. It is also essential for continuous clearance of dying cells, tissue remodeling, and acquisition of nutrients for some cells. Phagocytosis is a specific form of endocytosis initiated by recognition and binding of foreign particles by cell surface receptors, followed by their engulfment, and formation of phagosomes. Maturing phagosomes transform to phagolysosomes which destroy the pathogen through enzymes and toxic peroxides. Zymosan prepared from yeast cell wall (*Saccharomyces cerevisiae*), and consisting of protein-carbohydrate complexes is frequently used as a pathogen in phagocytosis assays. PromoKine's Phagocytosis Assay Kit (Green Zymosan) 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 receptor (TLR) ligands.
Quantity	100 assays
Kit Components	**Components** / **Quantity** / **Color Code** Phagocytosis Assay Buffer / 2 x 100 ml / NM Buffer Additive / 2 x 1 ml / Blue Green Zymosan / 600 µl / Green 10X Quenching Solution / 500 µl / Yellow
User Supplied Reagents & Equipment	• A 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. • Adherent or suspension cells capable of phagocytosis and appropriate media (e.g., JM774 or U937) • Stock solutions of effectors of interest (for example, Cytochalasin D, inhibitor of actin cytoskeletal rearrangement) • 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
Applications	• Rapid detection, quantification and validation of phagocytosis in convenient 96-well format • Tracking ligand internalization and screening for effectors of phagocytosis
Sample Type	• Cell culture: Adherent and suspension cells capable of phagocytosis
Storage and Reagents Preparation	• Store the entire kit at 4°C protected from light. Read the entire protocol before performing the assay. • **Phagocytosis Assay Buffer**: Upon arrival, combine one entire vial of Buffer Additive with one Phagocytosis Assay Buffer, mix well. Use sterile pipetting technique throughout the assay. • **Green Zymosan Suspension**: Before each use, equilibrate the suspension to room temperature and vortex gently for 5 seconds. • **Quenching Solution**: Dilute the content of the vial into 4.5 ml of 1x Phagocytosis Assay Buffer.
Assay Protocol	**1. Preparation of control and experimental wells**: Subculture cells capable of phagocytosis (adherent or suspension) in appropriate medium. Day before the experiment, determine viability of cells (see PromoKine's range of cell viability assays) and re-suspend cells to a concentration of 1 - 5 x 10^6 viable cells/ml. Seed 100 µl of cell culture per well omitting the negative control wells and incubate overnight at 37 °C, 5 % CO_2. Next day, change the media and proceed to the phagocytosis effector assay. Your experiment should always consist of parallel negative, positive and experimental wells respectively. **2. Phagocytosis effector assay**: 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. Aliquot 100 µl of media to each of the positive and 200 µl media to each

of the negative control wells respectively. Incubate for 1 hour at 37 °C, 5 % CO_2.

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

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

5. Sample preparation: Harvest the cells from each well into a separate tube and pellet for 5 minutes at 400 X g. Carefully aspirate off the media and gently re-suspend 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. Centrifuge for 5 minutes at 400 X g and repeat the washing step 3 more times. Finally, suspend the cells in 200 µl of ice cold Phagocytosis Assay Buffer and proceed to the preferred method of detection.

6. Detection: Cells can be analyzed by FACS, fluorescent microscopy or by scanning of all experimental and control wells in the plate reader at Ex/Em at 490/520 nm, respectively.

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.

a. For microscope and plate reader: Transfer 100 µl of each control and sample into a separate well and record the fluorescence. For fluorescent microscope: Control and experimental wells can be imaged directly in the plate.

b. For flow cytometry: In the flow cytometry compatible vessel mix 100 µl of cell suspension and 900 µl of the Phagocytosis Assay Buffer. Analyze immediately in the FL1 channel of flow cytometer equipped with laser capable of excitation at 488 nm.

7. Calculation: 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:

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

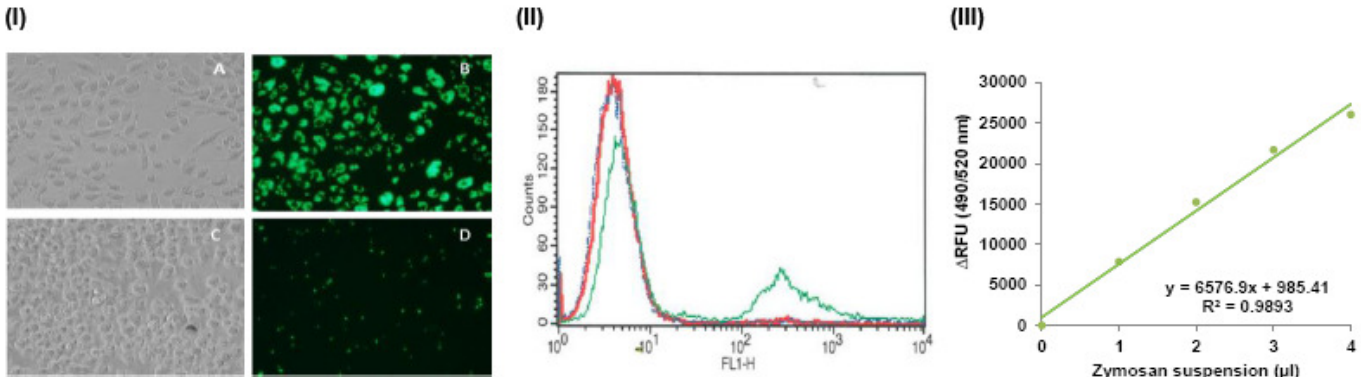

Figure: J774 macrophages were seeded overnight at 5 x 10^5 of viable cells/well. The next day the cells were pretreated with 20 µM Cytohalasin D for 1 h at 37°C prior to addition of 5 µl of Zymosan particles. Phagocytosis was conducted for 2 hours and the amount of engulfed Zymosan was determined as described in the Assay Protocol. (I) Inhibition of phagocytosis. Panel A and B: images of non-treated cells. Panel C and D: treatment with Cytohalasin D. (II) Flow cytometry plot. Red line: untreated control cells; green line: macrophages with engulfed Zymosan particles; blue line: inhibition of phagocytosis by Cytochalasin D. (III) Zymosan Standard curve.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40