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ab235900 Phagocytosis Assay Kit (Green *E. coli*)

For the measurement of phagocytosis in adherent or suspension cells.

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

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1. Overview

Phagocytosis Assay Kit (Green *E. coli*) (ab235900) utilizes heat-killed, fluorescently pre-labeled *E. coli* 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.

Prepare cells.

⇩

Change media and add cell culture media containing effector of interest.

⇩

Add *E. coli* slurry to all the wells.

⇩

Prepare Green *E. coli* Standard Curve.

⇩

Harvest cells by centrifugation and wash cell pellets three times in ice cold Assay Buffer 27 containing the effector of interest.

⇩

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.

2. Materials Supplied and Storage

All components in this kit are shipped on blue ice and are suitable for storage at 4°C, unless reconstituted. Upon receipt, immediately store kit at 4°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing 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)	Storage temperature (after prep)
Assay Buffer 27	2 x 100 mL	4°C or -20°C	4°C
Buffer Additive Solution	2 x 1 mL	4°C	4°C
Green *E. coli*	600 µL	4°C	4°C
10X Quenching Solution I	0.5 mL	4°C	4°C

PLEASE NOTE: Assay Buffer 27 was previously labelled as Assay Buffer XXVII and Phagocytosis Assay Buffer. The composition has not changed.

3. Materials Required, Not Supplied

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

- 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.
- 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.
- Adherent or suspension cells capable of phagocytosis (e.g., JM774 or U937).

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

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 Buffer Additive Solution

1. Ready to use as supplied.

5.2 Assay Buffer 27

1. Combine one entire vial of Buffer Additive Solution with one Assay Buffer 27, mix well.
2. Use sterile pipetting technique throughout the assay.

5.3 Green *E. coli*

1. Ready to use as supplied.
2. Before each use, equilibrate the suspension to room temperature and vortex gently for 5 seconds.

5.4 10X Quenching Solution I

1. Dilute the content of the vial into 4.5 mL of Assay Buffer 27.

6. 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.

6.1 Preparation of control and experimental wells:

1. Subculture cells capable of phagocytosis in appropriate medium.
2. 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.

6.2 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.

6.3 Phagocytosis of Green *E. coli*:

1. Add 5 µL of *E. coli* 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.

6.4 Green *E. coli* Standard Curve:

1. Add 0, 1, 2, 3 and 4 µL of Green *E. coli* slurry into a series of wells in 96-well plate.
2. Adjust the volume to 100 µL with Assay Buffer 27. 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.

6.5 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 Assay Buffer 27 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 Assay Buffer 27 and proceed to the preferred method of detection.

6.6 Detection:

1. 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.
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 Assay Buffer 27.
3. For **plate reader**: Transfer 100 µl of each control and sample into a separate well and record the fluorescence.
4. For **fluorescent microscope**: Control and experimental wells can be imaged directly in the plate.
5. For **flow cytometry**: Transfer 100 µL of cell suspension into a 900 µL of the Assay Buffer 27 in the flow cytometry compatible vessel. Analyze immediately in the FL1 channel of flow cytometer equipped with laser capable of excitation at 488 nm.

7. Data Analysis

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

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

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only**.

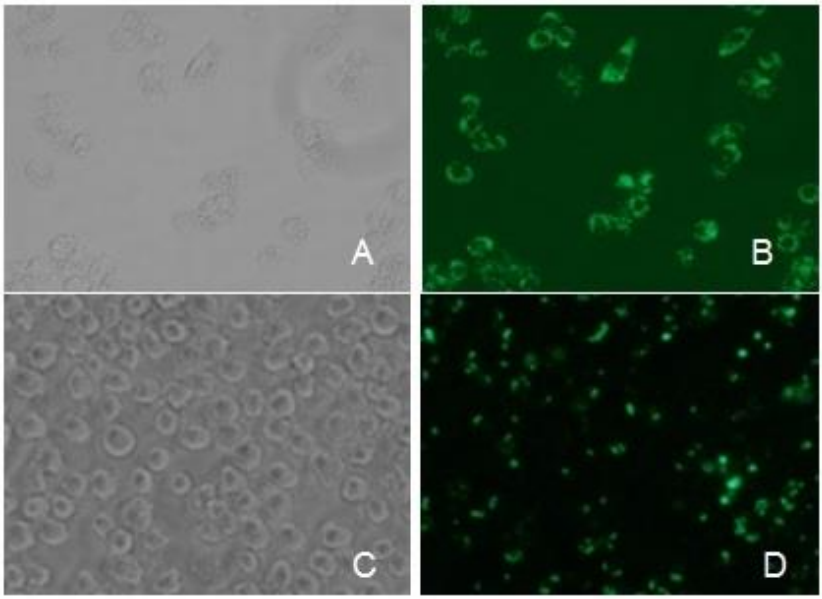

Figure 1. Inhibition of phagocytosis. J774 macrophages were seeded overnight at 5 x 10^5 of viable cells/well. The next day the cells were pretreated with 20 µM Cytochalasin D for 1 hour at 37°C prior to addition of 5 µL of *E. coli* particles. Phagocytosis was conducted for 2 hours and the amount of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

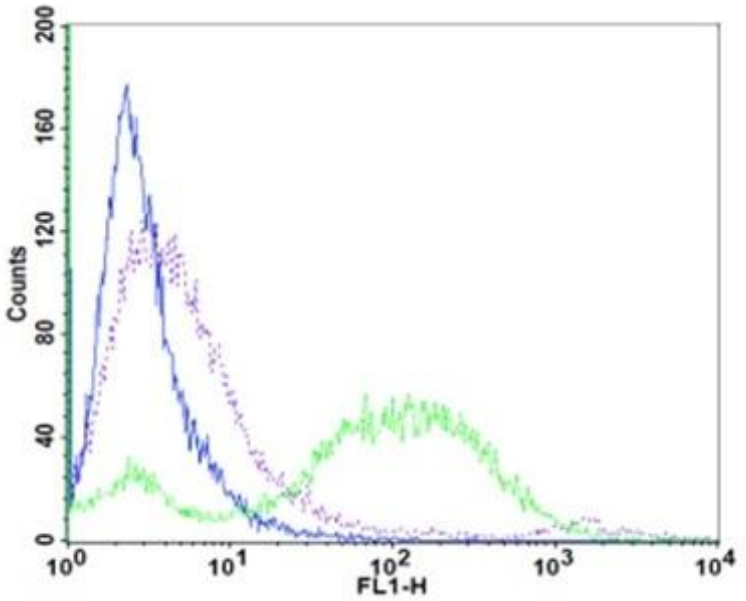

Figure 2. Flow cytometry plot. J774 macrophages were seeded overnight at 5 x 10^5 of viable cells/well. The next day the cells were pretreated with 20 µM Cytochalasin D for 1 hour at 37°C prior to addition of 5 µL of *E. coli* particles. Phagocytosis was conducted for 2 hours and the amount of engulfed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

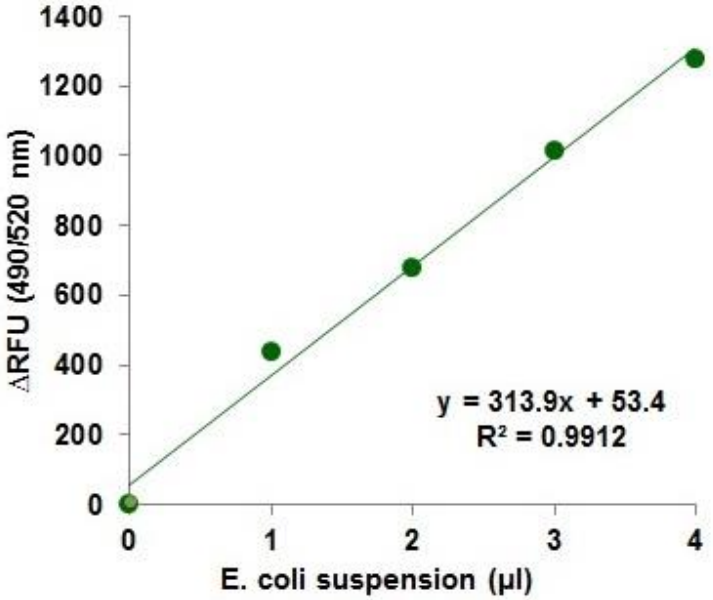

Figure 3. *E. coli* Standard curve.

10. 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)