Phagocytosis Assay Kit (IgG FITC)

Item No. 500290

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Customer Service 800.364.9897
Technical Support 888.526.5351
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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400291	Latex Beads-Rabbit IgG-FITC Complex	1 vial/150 µl	4°C
10009322	Cell-Based Assay Buffer Tablet	2 tablets	RT
400292	Trypan Blue (10X)	1 vial/500 µl	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorescence microscope or flow cytometer capable of measuring FITC fluorescence (ex/em 485 nm/535 nm)
2. For fluorescence microscopy: appropriate vessels for treating and observing cells (chamber slides or coverslips)
3. For flow cytometry: test tubes or 96 well v-bottom plates as appropriate for your flow cytometer
4. A source of phagocytic cells (such as human PBMCs, mouse bone marrow-derived macrophages, or cell lines like RAW 264.7 or THP-1)

INTRODUCTION

About This Assay

Cayman's Phagocytosis Assay Kit (IgG FITC) employs latex beads coated with fluorescently-labeled rabbit IgG as a probe for the measurement of the phagocytic process *in vitro*. The engulfed fluorescent beads can be detected using a fluorescence microscope, allowing kinetic studies of phagocytosis at the single-cell level. In addition, the flow cytometric readout provides the advantage of visualizing perturbations in phagocytosis on the population level and, when combined with antibody staining, of specific cell types within complex populations. This kit provides enough Latex Beads-Rabbit IgG-FITC Complex for up to 750 samples.

PRE-ASSAY PREPARATION

NOTE: The Latex Bead-Rabbit IgG-FITC Complex is light sensitive. Do not expose to direct intense light.

Reagent Preparation

1. **Assay Buffer Preparation**

   Dissolve each cell-based assay buffer tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. **Trypan Blue Quenching Solution Preparation**

   Prepare a trypan blue quenching solution by diluting the trypan blue stock solution (Item No. 400292) 1:10 in the assay buffer. Mix well to make sure there are no particles or flakes in the solution.

3. **Latex Beads-Rabbit IgG-FITC Complex**

   Ready to use as supplied. The beads have a 0.1 micron mean particle size.

ASSAY PROTOCOL

Adherent Cells

1. Plate the cells at a concentration such that they will be less than 80% confluent at treatment and allow to adhere.
2. Add latex beads-rabbit IgG-FITC complex (Item No. 400291) directly to your pre-warmed culture medium to a final dilution of 1:100 to 1:500.
3. Culture cells at 37°C for the period of time required for your experiment. Phagocytosis can begin within minutes of bead addition and continue for hours.
4. For fluorescence microscopy, uptake of beads can be visualized directly in culture with no additional washing steps. However, if staining with live/dead stains or antibodies to surface markers is desired, gentle washing with assay buffer will remove culture medium and unbound beads. Staining can be performed according to your lab's protocols, followed by visualization.
5. For flow cytometry, cells must be removed from the dish in which they are cultured by gentle scraping. Transfer the cells to FACS tubes or 96-well v-bottom plates for further staining or immediate flow cytometry.
6. To distinguish cells which have phagocytosed the beads from those simply binding the beads at the surface, a short (1-2 minute) incubation with trypan blue quenching solution, followed by a wash with assay buffer, will quench surface FITC fluorescence.

Suspension Cells

1. Suspend cells at a concentration of approximately 1-5 x 10^6 cells/ml in culture medium.
2. Place 100 µl of cells into each well of a 96-well v-bottom plate or each FACS tube.
3. Add latex beads-rabbit IgG-FITC complex directly to your pre-warmed culture medium to a final dilution of 1:100 to 1:500.
4. Incubate cells at 37°C for the period of time required for your experiment. Phagocytosis can begin within minutes of bead addition and continue for hours.
5. To assess the degree of phagocytosis, centrifuge the cells in the plate or tubes at 400 x g for five minutes, remove the supernatant, and resuspend the cells in 200-500 µl assay buffer. Flow cytometry can be performed immediately.
6. If further staining with antibodies to surface markers or live/dead dyes is required for your application, maintaining the cells on ice will prevent changes in the FITC fluorescence.
7. To distinguish cells which have phagocytosed the beads from those simply binding the beads at the surface, a short (1-2 minute) incubation with trypan blue quenching solution, followed by a wash with assay buffer, will quench surface FITC fluorescence.

PERFORMANCE CHARACTERISTICS

Flow Cytometry

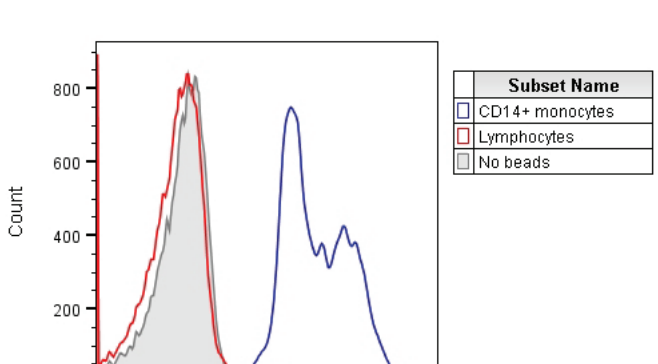

Figure 1. Human peripheral blood CD14+ monocytes phagocytose opsonized particles.
Fresh peripheral blood leukocytes were isolated and incubated in RPMI medium with a 1:400 dilution of latex beads-rabbit IgG-FITC complex or no beads (shaded) for four hours in a 96-well v-bottom plate. After pelleting, cells were resuspended in anti-human CD14-PE and incubated on ice for 20 minutes, then washed once with assay buffer and read on a flow cytometer. Using FlowJo software, CD14+ monocytes (blue) and lymphocytes (red) are gated and shown for FITC fluorescence.

Microscopy

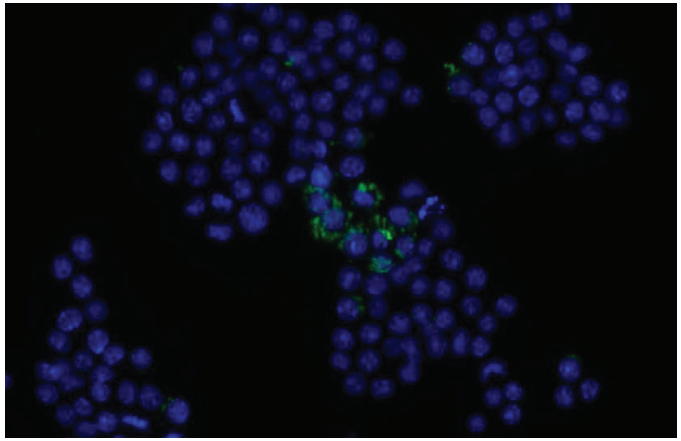

Figure 2. RAW 264.7 cells phagocytose opsonized particles.
RAW 264.7 cells (2 x 10^5 cells/ml) were plated on a 4-well chamber slide and allowed to adhere overnight. Latex beads-rabbit IgG-FITC complex was added directly to the culture medium at a 1:200 dilution and incubated at 37°C for two hours. Cells were gently washed with assay buffer twice, followed by counterstaining with 40 µM Hoechst 33342 for 10 minutes at 37°C. After two washes, cells were visualized at 20X magnification with a microscope.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Cells do not respond to treatment	A. Cells are from a late passage and may have lost the capacity to respond B. Cells are not healthy	A. Use cells at a low passage number B. Use only healthy cells
High background staining in all cells regardless of treatment	A. Inadequate washing B. Cells used in the experiment have tendency to attract the bead complex to the membrane	A. Perform washes with Assay Buffer B. Use Trypan Blue included in the kit to quench non-specific staining

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.