Phagocytosis Assay Kit (IgG PE)

Item No. 600540

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
600541	Latex Beads-Rabbit IgG-PE 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: 734-971-3640

Email: techserv@caymanchem.com

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 phycoerythrin fluorescence (ex/em 494 nm/575 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 PE) 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-PE Complex for up to 750 samples.

PRE-ASSAY PREPARATION

NOTE: The Latex Bead-Rabbit IgG-PE 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 IgG-PE 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-PE Complex (Item No. 600541) 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.

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-PE 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 PE fluorescence.

PERFORMANCE CHARACTERISTICS

Flow Cytometry

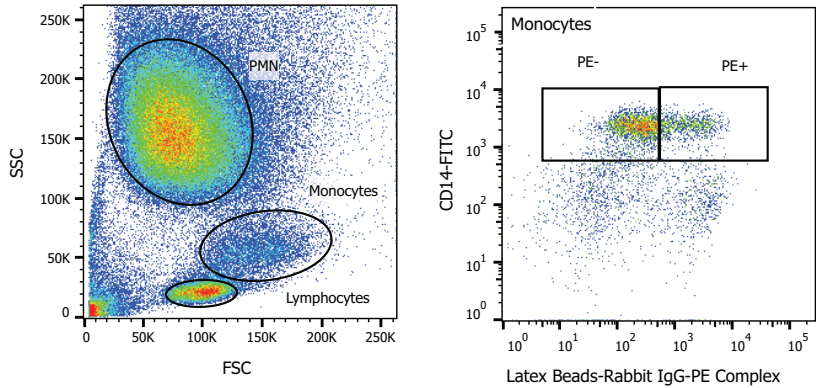

Figure 1. Peripheral blood monocytes (CD14+) phagocytose opsonized particles. Purified peripheral blood leukocytes were incubated with a 1:100 dilution of Latex Beads-Rabbit IgG-PE Complex for 1-3 hours. After washing excess beads out, cells were stained with anti-CD14-FITC antibody followed by flow cytometry. Monocytes were gated by forward/side scatter as shown in the left panel. The degree of PE uptake in the CD14+ population is shown in the right panel.

Microscopy

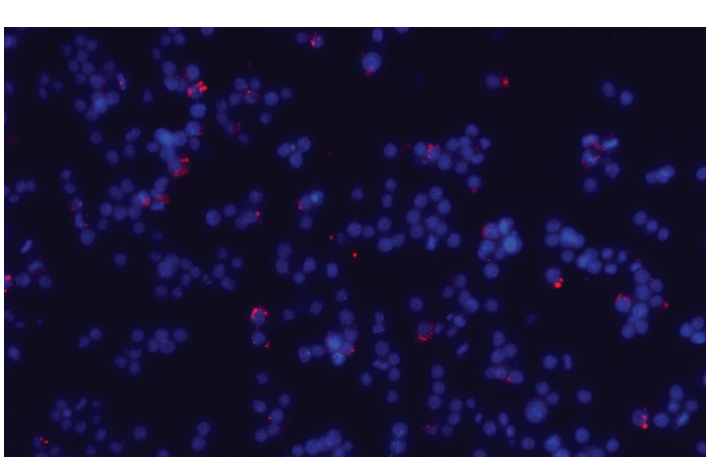

Figure 2. RAW264.7 cells take up IgG-coated latex beads. Cell-Based Assay Hoechst Dye (Item No. 600332)-stained murine macrophage-like RAW264.7 cells were incubated with a 1:100 dilution of Latex Beads-Rabbit IgG-PE Complex for 2 hours. After one wash with Assay Buffer, Hoechst (blue) and PE (red) fluorescence were imaged on a fluorescence microscope at 200X magnification and overlaid using Image J.

Appendix

Compatible viability dyes and nuclear counterstains (purchased separately):

1. **DAPI Viability Dye (Item No. 601361)**

   This vial contains 100 µl of DAPI in PBS. To use for fluorescence microscopy or flow cytometry, dilute 1:100 in PBS, pH 7.4, and add 100-500 µl per $1x10^5$ to $1x10^6$ cells. Assay immediately; dead cells fluoresce with excitation of 350-405 nm and emission around 450 nm.

2. **Cell-Based Assay Calcein AM (Item No. 400146)**

   This vial contains 50 µl of 1 mM calcein AM. To stain adherent cells for fluorescence microscopy prior to addition of latex beads, dilute to 1 µM in your warm culture medium and add to cells. Incubate for 30 minutes at 37°C, aspirate, and wash with Assay Buffer or medium. Visualize using a typical FITC filter set (max ex/em 494/520 nm) within 4 hours. Cells expressing P-gp or MRP multi-drug resistance proteins are not suitable for use with this dye as a counterstain.

3. **Cell-Based Assay Hoechst Dye (Item No. 600332)**

   This vial contains 50 µl of the cell permeable DNA dye Hoechst. To use as a nuclear counterstain for fluorescence microscopy, add to your culture medium to a final dilution of 1:2,500 to 1:5,000 prior to addition of latex beads. Incubate at 37°C for 15-30 minutes, aspirate and add medium with latex beads. Assay with a typical ultraviolet filter set (max ex/em 350/450 nm).

4. **RedDot™2 Viability Dye (Item No. 601282)**

   This vial contains 50 µl of the cell-impermeable DNA dye RedDot™2, a product of Biotium, Inc. To use for fluorescence microscopy or flow cytometry, dilute 1:200 in PBS, pH 7.4, and add 100-500 µl per $1x10^5$ to $1x10^6$ cells. Assay immediately; dead cells fluoresce with excitation of 633 nm and emission around 700 nm.

5. **DRAQ7™ (Item No. 19774)**

   This vial contains the cell impermeable dye DRAQ7™, a product of Biostatus. To use as a viability dye for fluorescence microscopy or flow cytometry, dilute 1:200 in PBS, pH 7.4, and add 100-500 µl per $1x10^5$ to $1x10^6$ cells. Assay immediately; dead cells fluoresce with excitation of 633 nm and emission around 700 nm.

6. **DRAQ7™ (Item No. 18781)**

   This vial contains the cell permeable DNA dye DRAQ5™, a product of Biostatus. To use as a nuclear counterstain for fluorescence microscopy, dilute 1:200 in PBS, pH 7.4, and add 100-500 µl per $1x10^5$ to $1x10^6$ cells. Assay immediately with a typical Cy5 filter set (max ex/em 647/681 nm).

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.