Phagocytosis Assay Kit (IgG-DyLight™ 633)

Item No. 601490

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
601491	Latex Beads-Rabbit IgG-DyLight™ 633 Complex	1 vial/100 µl	4°C
10009322	Cell-Based Assay Buffer Tablet	2 tablets	RT

NOTE: DyLight™ 633 is a product of Thermo Scientific.

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

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 far red fluorescence (ex/em 633 nm/700 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) and appropriate culture medium.

INTRODUCTION

About This Assay

Cayman's Phagocytosis Assay Kit (IgG-DyLight™ 633) 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-DyLight™ 633 Complex for up to 500 samples.

PRE-ASSAY PREPARATION

NOTE: The Latex Bead-Rabbit IgG-DyLight™ 633 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.

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-DyLight™ 633 Complex (Item No. 601491) directly to your pre-warmed culture medium to a final dilution of 1:100 to 1:500. The beads have a 0.1 µm mean particle size.
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. Aspirate the media and wash once with Assay Buffer. Add enough Assay Buffer to cover cells.
5. Fluorescence microscopy may be performed immediately, using a filter set which excites at 633 nm and detects emission around 700 nm.
6. 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 immediate flow cytometry.
7. **Optional:** If staining for viability or antibodies to surface markers is desired, staining can be performed according to your lab's protocols, followed by visualization by fluorescence microscopy or flow cytometry. Some compatible viability or nuclear dyes are listed in the Appendix on page 11.

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-DyLight™ 633 Complex (Item No. 601491) 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. 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 using a 633 nm laser and filter around 700 nm.
6. **Optional:** If further staining with antibodies to surface markers or viability dyes is required for your application, maintaining the cells on ice will prevent changes in the DyLight™ 633 fluorescence. Some compatible viability dyes are listed in the Appendix on page 11.

PERFORMANCE CHARACTERISTICS

Flow Cytometry

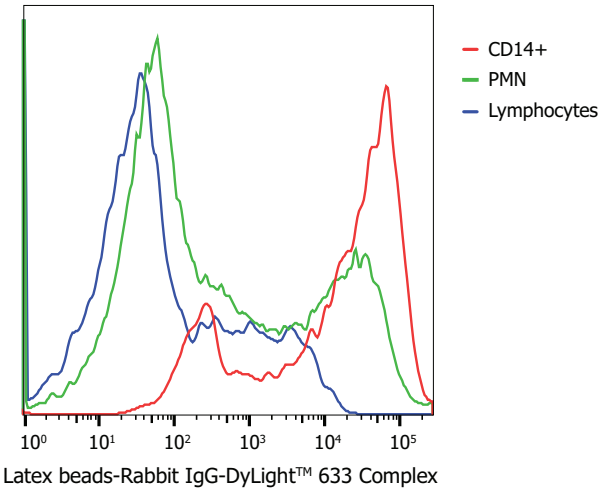

Figure 1. Peripheral blood monocytes (CD14+) phagocytose opsonized particles. Peripheral blood leukocytes were incubated with a 1:100 dilution of Latex Beads-Rabbit IgG-DyLight™ 633 Complex for 2 hours. Cells were stained with an anti-CD14 antibody and then subjected to flow cytometry. Neutrophils (PMN) and lymphocytes were identified by scatter and monocytes by CD14 expression, and phagocytosis by each subset was visualized in the far-red channel (633 nm excitation, 655-730 nm emission).

Microscopy

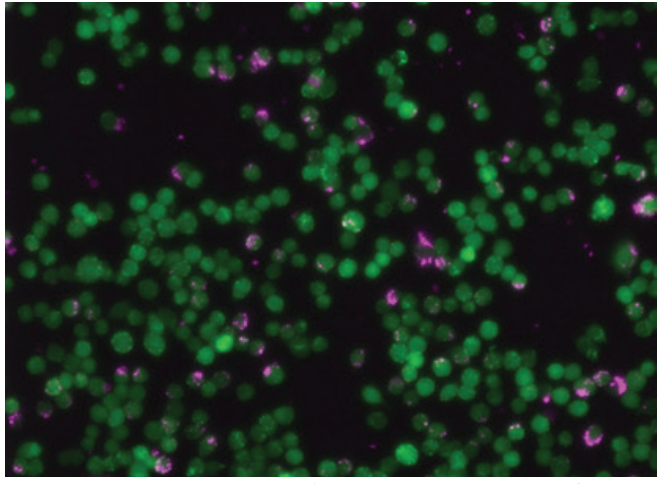

Figure 2. RAW 264.7 cells take up IgG-coated latex beads.
Calcein AM (Item No. 400146) stained murine macrophage-like RAW 264.7 cells were were plated at $2.5x10^5$ cells/well in a 24 well plate and incubated with a 1:100 dilution of Latex Beads-Rabbit IgG-DyLight™ 633 Complex for 2 hours. After one wash with Assay Buffer, calcein (green) and Dylight™ 633 (magenta) fluorescence were imaged on a fluorescence microscope at 200X magnification and overlaid using Image J.

RESOURCES

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 Assay Buffer, and add 100-500 µl per $1x10^5$ to $1x10^6$ pelleted cells. Assay immediately; dead cells fluoresce with excitation of 350-405 nm and emission around 450 nm.

2. **Cell-Based Assay 7-AAD Staining Stock Solution (1,000X) (Item No. 400201)**

   This vial contains 50 µl of 1000X 7-AAD. To use for fluorescence microscopy or flow cytometry, dilute 1:1,000 in Assay Buffer and add 100-500 µl per $1x10^5$ to $1x10^6$ pelleted cells. Assay immediately; dead cells fluoresce with excitation of 488 nm and emission of 650-700 nm.

3. **Cell-Based Propidium Iodide Solution (Item No. 10011234)**

   This vial contains 250 µl of Propidium Iodide solution. To use for fluorescence microscopy or flow cytometry, dilute 1:2,000 in Assay Buffer and add 100-500 µl per $1x10^5$ to $1x10^6$ pelleted cells. Assay immediately; dead cells fluoresce with excitation of 488 nm and emission around 650 nm.

4. **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.

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

   This vial contains 50 µl of the cell-permeable DNA dye Hoechst 33342. To use as a nuclear counterstain for fluorescence microscopy, dilute 1:2,500 in Assay Buffer, and add sufficient volume to cover cells. Incubate 10 minutes at room temperature and assay with a typical ultraviolet filter set (max ex/em 350/450 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 a bead-binding control sample: Treat cells with 10 µM cytochalasin D or incubate on ice to prevent uptake.

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.