Phagocytosis Assay Kit
(IgG-DyLight™ 405)

Item No. 601480

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA
Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity/Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>601481</td>
<td>Latex Beads-Rabbit IgG-DyLight™ 405 Complex</td>
<td>1 vial/100 µl</td>
<td>4°C</td>
</tr>
<tr>
<td>10009322</td>
<td>Cell-Based Assay Buffer Tablet</td>
<td>2 tablets</td>
<td>RT</td>
</tr>
</tbody>
</table>

NOTE: DyLight™ 405 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.

General Information

NOTE: 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 violet fluorescence (ex/em 405/450 nm). NOTE: DAPI filter sets may not provide sufficient excitation for DyLight™ 405.
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™ 405) 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™ 405 Complex for up to 500 samples.
NOTE: The Latex Bead-Rabbit IgG-DyLight™ 405 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.

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™ 405 Complex (Item No. 601481) 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. Aspirate the media and wash once with Assay Buffer. Add enough Assay Buffer to cover the cells.

5. Fluorescence microscopy may be performed immediately, using a filter set which excites at 405 nm and detects emission around 450 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. Maintaining the cells on ice will prevent changes in the DyLight™ 405 fluorescence. Some compatible viability dyes and nuclear counterstains are listed in the Appendix on page 11.
Suspension Cells

1. Suspend cells at a concentration of approximately $1-5 \times 10^6$ cells/ml in warm 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™ 405 Complex (Item No. 601481) 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 405 nm laser and filter around 450 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™ 405 fluorescence. Some compatible viability dyes are listed in the Appendix on page 11.

Flow Cytometry

Peripheral blood monocytes (CD14+) phagocytose opsonized particles. Peripheral blood leukocytes were incubated with a 1:100 dilution of Latex Beads-Rabbit IgG-DyLight™ 405 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 violet channel (405 nm excitation, 450 emission).

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™ 405 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 violet channel (405 nm excitation, 450 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 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™ 405 Complex for 2 hours. After one wash with Assay Buffer, calcein (green) and Dylight™ 405 (blue) 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. **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.

2. **Cell-Based Assay 7-AAD Staining Stock Solution (1,000X) (Item No. 400201)**
   - This vial contains 50 µl of 1,000X 7-AAD. To use for fluorescence microscopy or flow cytometry, dilute 1:1000 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. **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 Assay Buffer, and add 100-500 µl per 1x10^5 to 1x10^6 pelleted cells. Assay immediately; dead cells fluoresce with excitation of 633 nm and emission around 700 nm.

4. **DRAQ7™ (Item No. 19774)**
   - This vial contains the cell impermeable dye DRAQ7™, a product of Biostatus, Ltd. To use as a viability dye for fluorescence microscopy or flow cytometry, dilute 1:200 in Assay Buffer, and add 100-500 µl per 1x10^5 to 1x10^6 pelleted cells. Assay immediately; dead cells fluoresce with excitation of 633 nm and emission around 700 nm.
5. **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 within 4 hours. Cells expressing P-gp or MRP multi-drug resistance proteins are not suitable for use with this dye as a counterstain.

6. **DRAQ5™ (Item No. 18781)**

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

### Troubleshooting

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<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
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| 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. |

### Warranty and Limitation of Remedy

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