Macrophage (mouse) Elicitation Kit

Item No. 601740

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

Kit will arrive packaged as a 4°C kit. After opening kit, store individual components as stated below.

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity/Size</th>
<th>Storage</th>
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</thead>
<tbody>
<tr>
<td>601741</td>
<td>Thioglycollate Broth Solution</td>
<td>1 vial/5 ml</td>
<td>4°C</td>
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<tr>
<td>10009322</td>
<td>Cell-Based Assay Buffer Tablet</td>
<td>1 tablet</td>
<td>RT</td>
</tr>
<tr>
<td>601742</td>
<td>Anti-CD11b PE Test Reagent</td>
<td>1 vial/120 µl</td>
<td>4°C</td>
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<tr>
<td>601743</td>
<td>Anti-F4/80 FITC Test Reagent</td>
<td>1 vial/120 µl</td>
<td>4°C</td>
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</table>

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
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 in the Materials Supplied section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied
1. Mice, 6-12 weeks of age.
2. Ethanol, 70%.
3. Syringes, 5 ml.
4. Hypodermic needles, 18-21G x 1”.
5. Swinging-bucket tabletop centrifuge (e.g., Sorvall® RT-6).
6. Conical polypropylene centrifuge tubes, 15 ml.
7. Polypropylene test tubes, 12 x 75 nm.
Background

Macrophages are a key cell type in innate immunity, representing a first line of defense against infections in tissues. They are long-lived tissue resident cells, professional phagocytes capable of ingesting and processing many varieties of pathogens, tumor cells, and dying cells as well as coordinating the immune response to these potential stimuli. Tissue resident macrophages represent a diverse cell type, acutely tuned to the tissue in which they reside and its unique requirements. They express a variety of receptors for detecting both pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Upon sensing these stimuli, macrophages secrete cytokines and chemokines that direct the response to the specific stimulus, recruiting the appropriate immune cells to eliminate the danger. Like many finely-tuned systems, they can be subverted to have pathogenic effects as well, as in the cases of pro-oncogenic tumor-associated macrophages and inflammatory macrophages found in atherosclerotic plaques.

About This Assay

Macrophages are long-lived tissue resident cells that function to ingest and kill invading pathogens and to direct the ensuing immune responses. Cayman’s Macrophage (mouse) Elicitation Kit provides the reagents necessary for eliciting macrophages to the peritoneal cavities of up to five mice and the fluorochrome-labeled antibodies required to assess the populations recovered by flow cytometry. This kit will be useful to immunologists interested in studying monocyte/macrophage trafficking as well as those requiring a source of macrophages for downstream applications.
Performing the Assay

NOTE: Peritoneal-resident macrophages can be extracted from the peritoneal cavities of mice without the injection of thioglycollate broth, but more cells will be collected with elicitation. The choice of whether to elicit depends on your experimental requirements.

Elicit and Collect Peritoneal Cells

1. Using institutionally-approved methods, inject 1 ml Thioglycollate Broth Solution into the peritoneal cavity of each mouse. House the mice normally for 72 hours after injection to allow optimal infiltration of macrophages into the peritoneal cavity.

2. Euthanize the mouse using an institutionally-approved method.

3. Immobilize the mouse on a dissecting board or other suitable work surface and clean the abdominal fur with 70% ethanol.

4. Optional: Make a shallow ventral midline incision through the abdominal skin with scissors (point up) taking care to avoid puncturing the transparent peritoneal wall below. Retract the abdominal skin to either side revealing the intact peritoneum.

5. Using a 5 ml syringe fitted with a 18-21G x 1” hypodermic needle, fill with 5 ml Assay Buffer. Inject the entire 5 ml into the peritoneal cavity with the bevel of the needle facing up. Take care to avoid puncturing the intestines.

6. Gently massage the peritoneal cavity to dislodge adherent cells. Rotate the syringe 180 degrees so that the bevel faces down and gently withdraw the fluid from the peritoneum. Gently pulling up, so that the needle forms a tent of the peritoneum, will help avoid aspirating abdominal fat or other organs. Move the needle to a new location if it catches a piece of fat or tissue. It should be possible to recover more than 4 ml of the injected 5 ml volume.
Flow Cytometric Analysis of Isolated Macrophages

*NOTE: Should the isolated macrophages be intended for culture, care should be taken to maintain sterile conditions.*

1. Transfer peritoneal exudate to a sterile conical tube.
2. Centrifuge at 250 x g for 5 minutes.
3. Remove the supernatant (may be reserved for other downstream applications such as ELISA), and resuspend the cell pellet in 5 ml Assay Buffer.
4. Count the cells and resuspend to 1 x 10^6 cells/ml in Assay Buffer.
5. In a clean polypropylene test tube, add 100 µl of the cell suspension (reserving the remaining cells on ice for your downstream applications). Be sure to prepare tubes for single antibody staining controls.
6. Centrifuge at 250 x g for 5 minutes.
7. Remove supernatant and resuspend pellet in 100 µl of the Staining Mix prepared on page 8.
8. Incubate on ice for 20 minutes.
9. Add 1 ml of Assay Buffer and centrifuge for five minutes at 250 x g.
10. Remove supernatant and resuspend pellet in 100-500 µl assay buffer.
11. Analyze immediately by flow cytometry. An example of cell populations recovered is shown in Figure 1, on page 11.
12. Further enrichment of macrophages from the peritoneal exudate may be carried out by adherence to cell culture plates or glass coverslips. To accomplish this, plate the cells in cell culture medium at 1 x 10^6 cells/ml for 1-2 hours at 37°C. Gently wash 3 times with warm PBS, and then proceed to your downstream application.

**Figure 1.** Flow cytometric analysis of peritoneal exudate macrophages. Peritoneal exudate was obtained from a naïve mouse (Panel A) and a thioglycollate-injected mouse (72 hours; Panel B) and stained as described in this kit booklet. Distribution of F4/80 and CD11b on resident and infiltrating monocyte/macrophage populations is indicated.
Figure 2. **Elicited peritoneal macrophages take up labeled dextran.** Peritoneal exudate was obtained from a thioglycollate-injected mouse (72 hours) as described in this kit booklet. These cells were plated and allowed to adhere, then treated with labeled dextran. Following overnight incubation, cells were stained with Hoechst dye (Item No. 600332) and 10X images were captured using Biotek's Cytation™ 5 Cell Imaging Multi-Mode Reader. Hoechst is depicted as blue and dextran as red in the image (Panel A) and digitally zoomed image (Panel B).

### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
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<tbody>
<tr>
<td>Peritoneal lavage cloudy or full of particulate matter</td>
<td>Intestines were punctured during injection or lavage</td>
<td>Discard and treat another mouse.</td>
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</tbody>
</table>

### References


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