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

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

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

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

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.
8. Flow cytometer with a 488 nm laser line.

INTRODUCTION

Background

Macrophages are a key cells to 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.[1] 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.

Because macrophages are associated with specific tissues and have different characteristics based on tissue origin, they can be rather difficult to study. A source of primary macrophages is a valuable tool and mouse peritoneal exudate is a commonly used source.[2] To obtain the peritoneal exudate, a volume of liquid is injected into the peritoneal cavity and withdrawn, capturing any cells not closely associated with an organ. A small number of macrophages normally reside in the peritoneal cavity. As shown by flow cytometry, these cells mostly highly express CD11b and the antigen recognized by the F4/80 antibody and are relatively large, though a distinct subset are smaller and express lower amounts of both markers. With induction of sterile inflammation in the peritoneal cavity using, for instance, Brewer's thioglycollate broth, monocytes are recruited into the peritoneal cavity from the circulation and differentiate into macrophage-like cells over the course of a few days. Thioglycollate elicitation should increase the cell number recovered from about 0.5-1 x 10^6 to about 5-10 x 10^6 per mouse. These infiltrating cells resemble the smaller resident population in size and surface marker expression.[3] Therefore, this method is not a perfect representation of resident macrophages, but elicited cells will have many functional similarities with resident macrophages.

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.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Thioglycollate Broth Solution**

   Open Thioglycollate Broth Solution (Item No. 601741) and use under sterile conditions. Warm to room temperature prior to injection. Enough thioglycollate broth is provided to perform five 1 ml injections, sufficient for five mouse preparations. Any unused thioglycollate broth can be stored at 4°C for up to 12 months.

2. **Assay Buffer**

   Dissolve one Assay Buffer Tablet (Item No. 10009322) in 100 ml water. Sterile filter and store at room temperature for up to one year.

3. **Staining Mix**

   Anti-CD11b PE Test Reagent (Item No. 601742) and Anti-F4/80 FITC Test Reagent (Item No. 601743) are provided as 10X stock reagents. For each sample to be tested, add 10 µl of each antibody to 80 µl Assay Buffer. For compensation controls, 10 µl of one antibody should be added to 90 µl Assay Buffer. Enough antibody solution has been provided to stain 12 samples for flow cytometry.

ASSAY PROTOCOL

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.

ANALYSIS

Performance Characteristics

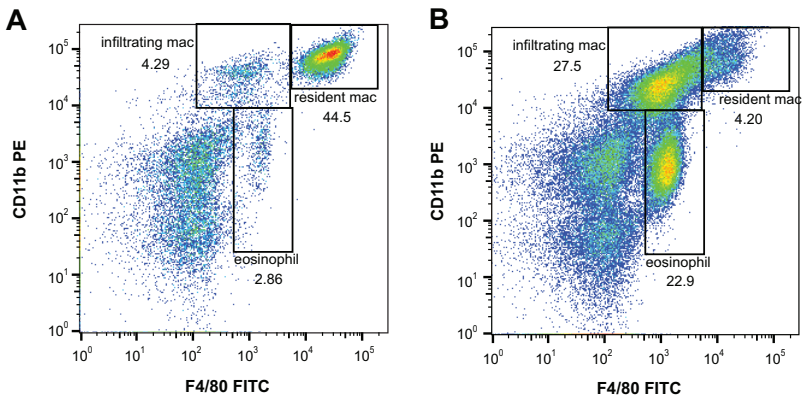

Figure 1. Flow cytometric analysis of peritoneal exudate macrophages. Peritoneal exudate was obtained from a naïve mouse (*Panel A*) and a thioglycollate-injected mouse (72 hours; *Panel B*) and stained as described in this kit booklet. Distribution of F4/80 and CD11b on resident and infiltrating monocyte/macrophage populations is indicated.

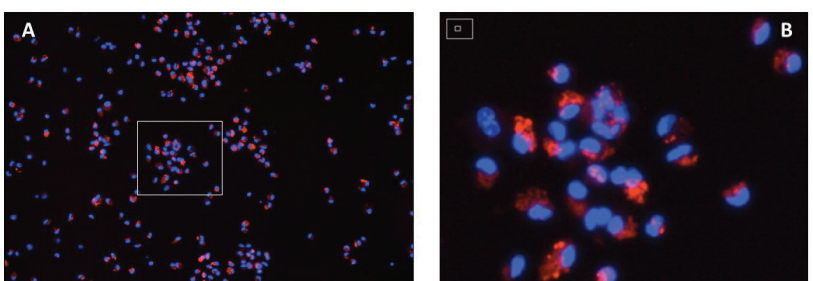

Figure 2. Elicited peritoneal macrophages take up labeled dextran. Peritoneal exudate was obtained from a thioglycollate-injected mouse (72 hours) as described in this kit booklet. These cells were plated and allowed to adhere, then treated with labeled dextran. Following overnight incubation, cells 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*).

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Peritoneal lavage cloudy or full of particulate matter	Intestines were punctured during injection or lavage	Discard and treat another mouse.

References

1. Gordon, S., Plüddemann, A., and Martinez-Estrada, F. Macrophage heterogeneity in tissues: Phenotypic diversity and functions. *Immunol. Rev.* **262(1)**, 36-55 (2014).
2. Ray, A. and Dittel, B.N. Isolation of mouse peritoneal cavity cells. *J. Vis. Exp.* **28(35)** (2010).
3. Ghosn, E.E., Cassado, A.A., Govoni, G.R., *et al.* Two physically, functionally, and developmentally distinct peritoneal macrophage subsets *Proc. Nat. Acad. Sci. USA* **107(6)**, 2568-2573 (2010).

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.