Easy Isolation of F4/80-Positive Macrophages from Mouse Tissues

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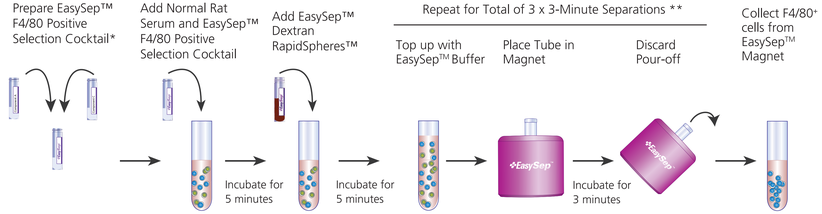
INTRODUCTION

Macrophages are important immunomodulators and also have critical roles in the development and homeostasis of tissues and organs. Our understanding of macrophages is constantly evolving and expanding, as they are actively studied in many areas of research, including infectious diseases, wound healing, and tumor immunology. Adding to the complexity of macrophage research, subsets of macrophages can be identified throughout the body with diverse phenotypes and functions. Furthermore, macrophage frequency can be highly variable across different tissues, presenting a challenge to obtain highly pure macrophages. To address these challenges, we have developed a simple method using the EasySep™ technology to isolate macrophages by targeting F4/80, a well-established mouse macrophage marker. Protocols have been optimized to accommodate a range of sample sizes and various tissue sources, including mouse peritoneal lavage fluid, lung, and spleen. EasySep™-isolated F4/80-positive macrophages are functional, as demonstrated by their ability to uptake FITC-dextran and secrete inflammatory mediators after TLR4 and TLR7 stimulation. Overall, the EasySep™ Mouse F4/80 Positive Selection Kit enables simple isolation of F4/80-positive macrophages in under 25 minutes, streamlining the experimental workflows for researchers studying macrophage biology.

METHODS



*Mix EasySep™ Mouse F4/80 Positive Selection Component A and B at a 1:1 ratio for peritoneal lavage or 1:2 ratio for lung and spleen.
**3 x 3-minute for peritoneal lavage, 3 x 5-minute for lung, and 4 x 3-minute separation for spleen.

FIGURE 1. EasySep™ Mouse F4/80 Positive Selection Protocol

Summary

- EasySep™ Mouse F4/80 Positive Selection Kit enables the isolation of F4/80-positive macrophages from various tissues in under 25 minutes.
- Up to 95% F4/80 purity can be achieved from peritoneal lavage and lung tissues, and 90% from splenoytes.
- The isolation protocol can accommodate a range of sample sizes and tissue sources.
- EasySep™-isolated macrophages are functional.

RESULTS

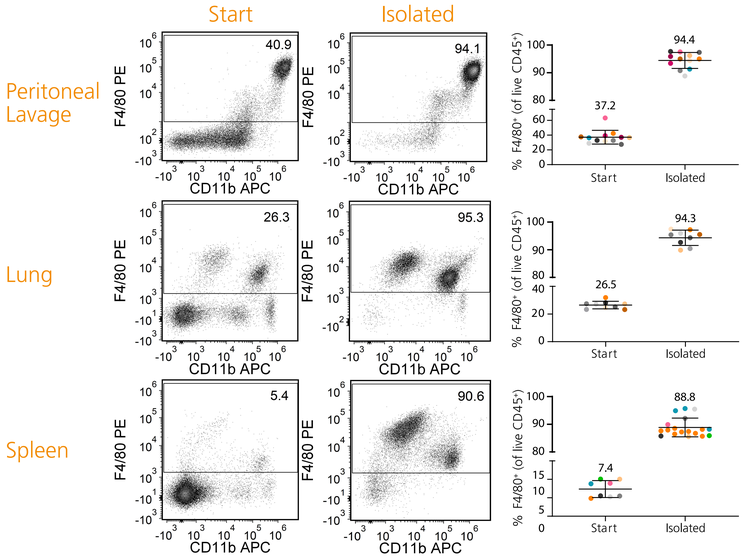


FIGURE 2. F4/80 Purity Before and After EasySep™ Isolation. F4/80-positive cells were isolated from various tissues using the EasySep™ (purple) Magnet. Peritoneal lavage, lung, and spleen samples were adjusted to 2.5 x 10^7 cells/mL, 5 x 10^7 cells/mL, and 1 x 10^8 cells/mL, respectively prior to EasySep™ isolation. F4/80 purity within the viable (DRAQ7™-negative) CD45-positive cell population was assessed by flow cytometry. Graph shows mean purity +/- SD from peritoneal lavage (n=12), lung (n=9), and spleen (n=18). Each dot represents an independent separation, and each color represents an independent start sample.

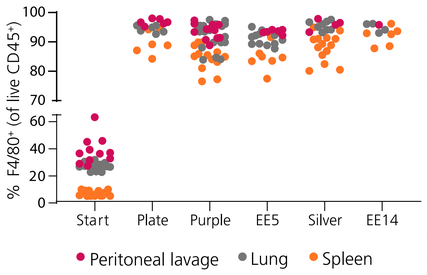


FIGURE 3. Compatibility of F4/80 Positive Selection Protocol with Various EasySep™ Magnets. F4/80-positive cell isolation from peritoneal lavage, lung and spleen tissues using EasyPlate™ (plate), EasySep™ (purple), "The Big Easy" EasySep™ (silver), and EasyEights™ (EE) Magnets. Peritoneal lavage, lung, and spleen samples were adjusted to 2.5 x 10^7 cells/mL, 5 x 10^7 cells/mL, and 1 x 10^8 cells/mL, respectively prior to EasySep™ isolation. Data includes isolations performed over multiple volumes.

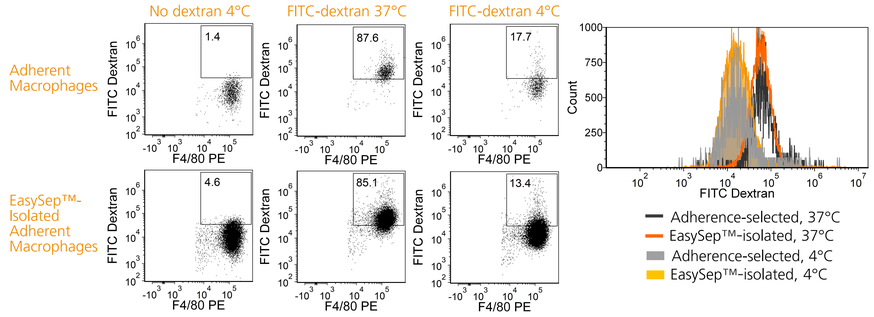


FIGURE 4. FITC-Dextran Uptake by F4/80-Positive Peritoneal Macrophages After EasySep™ Isolation. Macrophages were isolated from peritoneal lavage of naïve C57BL/6 mice by plate adherence after 24 hours in culture, or first selected by F4/80 using EasySep™ prior to plating. Macrophages were cultured in the presence or absence of FITC-dextran (70 kDa, 0.5 mg/mL) at 4°C or 37°C for 45 minutes. Gating on F4/80+ CD11b+ macrophages, FITC-dextran uptake by macrophages was analyzed by flow cytometry. Data shown are representative of two independent experiments.

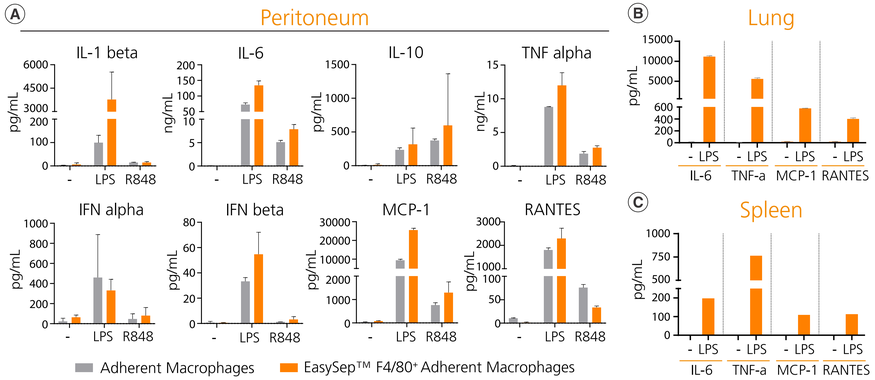


FIGURE 5. EasySep™-Isolated F4/80-Positive Macrophages Produce Cytokines and Chemokines Upon Stimulation. (A) Macrophages were isolated from peritoneal lavage of naïve C57BL/6 mice by plate adherence after 24 hours in culture (grey bar), or first selected by F4/80 using EasySep™ prior to plating (orange bar). MSD multiplex cytokine assay was performed on macrophage cell culture supernatant after 48 hours of stimulation with or without TLR4 agonist lipopolysaccharide (LPS) at 100 ng/mL, or TLR7 agonist R848 at 25 ng/mL. EasySep™-isolated macrophages from **(B)** lung and **(C)** spleen were cultured in the presence or absence of LPS for 48 hours before cytokine analysis by MSD multiplex assay. Data shown as mean +/- SD (A: n=1 duplicates; B: n=1 duplicates; C: n=1).