# EasySep™ Mouse CD11c Positive Selection Kit

Positive Selection

Catalog #18758

For processing 2 x 10^9 cells

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## Description

Isolate highly purified CD11c+ cells from mouse splenocytes or other single-cell suspensions by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- Up to 98% purity
- No columns required

This kit targets CD11c+ cells for positive selection with an antibody recognizing the CD11c surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture or DNA/RNA extraction.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| EasySep™ Mouse CD11c PE Labeling Reagent | 18758C.2 | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS with 0.1% BSA and < 0.1% sodium azide |
| EasySep™ PE Selection Cocktail | 18154 | 3 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS with 0.1% BSA |
| EasySep™ Magnetic Nanoparticles Positive Selection | 18150 | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water. |
| EasySep™ Mouse FcR Blocker | 18730 | 1 x 0.2 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS with 0.1% BSA and < 0.1% sodium azide |

BSA - bovine serum albumin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

SPLEEN

Use Spleen Dissociation Medium (Catalog #07915). Refer to this product's Product Information Sheet (Document #29636) for detailed information on the recommended protocol.

BONE MARROW

Flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23 gauge needle. Disperse clumps by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer.

Centrifuge at 300 x g for 10 minutes and resuspend cells at 0.5 - 2 x 10^6 cells/mL in RPMI 1640 (Catalog #36750) containing 10% fetal bovine serum (FBS), 2 mM L-Glutamine (Catalog #07100), 5 µM 2-mercaptoethanol, 10 ng/mL Mouse Recombinant GM-CSF (Catalog #78017), and 10 ng/mL Mouse Recombiinant IL-4 (Catalog #78047), or 200 ng/mL Mouse Recombinant Flt3/Flk-2 Ligand (Catalog 78011).

Plate 20 mL per 150 mm Petri dish and incubate at 37°C for 5 days. Collect non-adherent cells, wash once, and resuspend at 1 x 10^8 cells/mL (EasySep™) or 1.5 x 10^8 cells/mL (RoboSep™) in recommended medium.

## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

# Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Mouse CD11c Positive Selection Kit Protocol**

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS |  **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)**  |
| 1 | **Prepare sample at the indicated cell concentration within the volume range.** | 1 x 10^8 cells/mL<br>0.1 - 2 mL<br>NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL | 1 x 10^8 cells/mL<br>0.5 - 6 mL<br>NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.5 mL |
| | **Add sample to required tube.** | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352058) | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 2 | **Add FcR blocker to sample.†** | 10 µL/mL of sample | 10 µL/mL of sample |
| 3 | **Add Labeling Reagent to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 15 minutes | RT for 15 minutes |
| 4 | **Add Selection Cocktail to sample.** | 100 µL/mL of sample | 100 µL/mL of sample |
| | **Mix and incubate.** | RT for 15 minutes | RT for 15 minutes |
| 5 | **Mix Magnetic Particles.** | Pipette up and down more than 5 times | Pipette up and down more than 5 times |
| 6 | **Add Magnetic Particles to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 10 minutes | RT for 10 minutes |
| 7 | **Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.** | Top up to 2.5 mL | • Top up to 5 mL for samples < 2 mL<br>• Top up to 10 mL for samples ≥ 2 mL |
| | **Place the tube (without lid) into the magnet and incubate.** | • For spleen cells: RT for 5 minutes<br>• For cultured cells: RT for 10 minutes | • For spleen cells: RT for 5 minutes<br>• For cultured cells: RT for 10 minutes |
| 8 | **Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.** | Discard supernatant | Discard supernatant |
| 9 | **Repeat steps as indicated.** | • For spleen cells: Steps 6 and 7, three more times (total of 4 x 5-minute separations)<br>• For cultured cells: Steps 6 and 7, one more time (total of 2 x 10-minute separations) | • For spleen cells: Steps 6 and 7, three more times (total of 4 x 5-minute separations)<br>• For cultured cells: Steps 6 and 7, one more time (total of 2 x 10-minute separations) |
| 10 | **Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.** | Isolated cells are ready for use | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)

† Use of the Mouse FcR Blocker may prevent subsequent attempts at cross-linking FcγRIII/II (CD16/32) molecules on the surface of selected cells to trigger signaling through these receptors (Siragam et al.). It may therefore be desirable to omit the EasySep™ Mouse FcR Blocker addition to the cell suspension for such studies.

* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

# Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

**Table 2. RoboSep™ Mouse CD11c Positive Selection Kit Protocol**

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000)  |
|---|---|---|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 1.5 x 10^8 cells/mL<br>0.3 - 4.5 mL<br>NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.3 mL |
| | Add sample to required tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 2 | Add FcR blocker to sample.† | 10 µL/mL of sample |
| 3 | Select protocol. | Spleen:<br>• For samples ≤ 3 mL use:<br>Mouse CD11c Positive Selection (spleen DC) 18758 - small volume<br>• For samples ≥ 3 mL use:<br>Mouse CD11c Positive Selection (spleen DC) 18758 - large volume<br>Cultured Bone Marrow:<br>• For samples ≤ 3 mL use<br>Mouse CD11c Positive Selection (cultured DC) 18758 - small volume<br>• For samples ≥ 3 mL use<br>Mouse CD11c Positive Selection (cultured DC) 18758 - large volume |
| 4 | Mix Magnetic Particles. | Pipette up and down more than 5 times |
| 5 | Load the carousel. | Follow on-screen prompts |
| | Start the protocol. | Press the green “Run” button |
| 6 | Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube. | Isolated cells are ready for use |

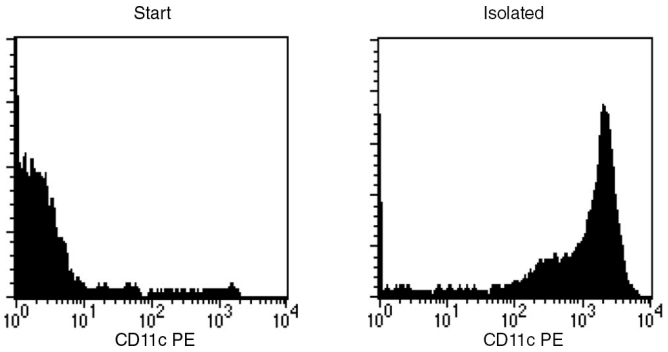
† Use of the Mouse FcR Blocker may prevent subsequent attempts at cross-linking FcγRIII/II (CD16/32) molecules on the surface of selected cells to trigger signaling through these receptors (Siragam et al.). It may therefore be desirable to omit Mouse FcR Blocker addition to the cell suspension for such studies.

## Notes and Tips

ASSESSING PURITY

The positively selected cells have already been PE-labeled so the purity can be assessed directly by flow cytometry.

## Data




Starting with mouse splenocytes, the CD11c+ cell content of the isolated fraction typically ranges from 87 - 98%. In the example above, the purity of the start and final isolated fractions are 7.2% and 93.1%, respectively.

## References

Siragam V et al. (2006) Intravenous immunoglobulin ameliorates ITP via activating Fc gamma receptors on dendritic cells. Nat Med 12(6): 688–92.