EasySep™

Positive Selection

Catalog #18751

# EasySep™ Mouse CD90.2/Thy 1.2 Positive Selection Kit

For processing 2 x 10^9 cells

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## Description

Isolate highly purified CD90.2+ 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 99% purity
- No columns required

This kit targets CD90.2+ cells for positive selection with antibodies recognizing the CD90.2 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 CD90.2 PE Labeling Reagent | 18751C.1 | 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, 0.1% BSA, and < 0.1% sodium azide. |
| EasySep™ PE Positive Selection Cocktail | 18154 | 2 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, and 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. |

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

Disrupt spleen in recommended medium. Remove clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing the cells for separation.

## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (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 CD90.2/Thy 1.2 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 - 2.5 mL<br>NOTE: For samples containing < 1 x 10^7 cells, resuspend to 0.1 mL | 1 x 10^8 cells/mL<br>0.25 - 8.5 mL<br>NOTE: For samples containing < 2.5 x 10^7 cells, resuspend to 0.25 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 Labeling Reagent to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 15 minutes, protect from light | RT for 15 minutes, protect from light |
| 3 | **Add Selection Cocktail to sample.** | 100 µL/mL of sample | 100 µL/mL of sample |
| | **Mix and incubate.** | RT for 15 minutes, protect from light | RT for 15 minutes, protect from light |
| 4 | **Mix Magnetic Particles.** | Pipette up and down more than 5 times | Pipette up and down more than 5 times |
| 5 | **Add Magnetic Particles to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 10 minutes, protect from light | RT for 10 minutes, protect from light |
| 6 | **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.** | RT for 5 minutes | RT for 5 minutes |
| 7 | **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 |
| 8 | **Repeat steps as indicated.** | Steps 6 and 7, two more times<br>(total of 3 x 5-minute separations) | Steps 6 and 7, two more times<br>(total of 3 x 5-minute separations) |
| 9 | **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)

* 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 CD90.2/Thy 1.2 Positive Selection Kit Protocol**

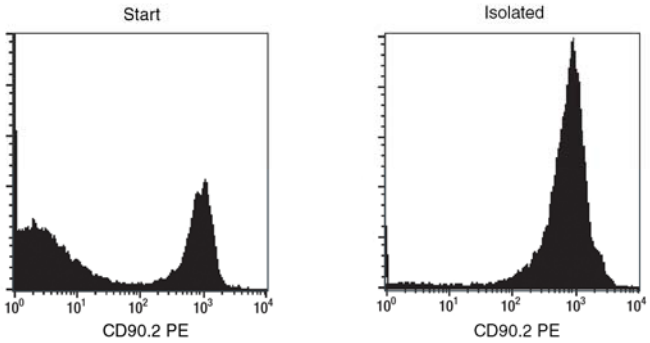
| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000)  |
|---|---|---|
| 1 | **Prepare sample at the indicated cell concentration within the volume range.** | 1 x 10^8 cells/mL<br>0.25 - 8 mL<br>NOTE: For samples containing < 2.5 x 10^7 cells, resuspend to 0.25 mL |
| | **Add sample to required tube.** | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 3 | **Select protocol.** | Mouse CD90.2 (Thy-1.2) Positive Selection 18751-high purity |
| 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 |

## 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 CD90.2+ cell content of the isolated fraction typically ranges from 96 - 99%. In the above example, the purities of the start and final isolated fractions are 35.9% and 96.4%, respectively.