

EasySep™ Mouse CD8+ T Cell Isolation Kit



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For processing 1 x 10⁹ cells

Catalog #19853

Catalog #19853RF RoboSep™

Negative Selection

Document #10000003748 | Version 03

Description

Isolate untouched and highly purified CD8+ T cells from mouse splenocytes by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- Up to 95% purity
- No column required
- Untouched, viable cells

This kit targets non-CD8+ T cells for removal with biotinylated antibodies recognizing specific cell surface markers. Unwanted cells are labeled with biotinylated antibodies and streptavidin-coated magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, and cell-based experiments.

Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|-------------|------------|-------------------------------------|--|---|
| EasySep™ Mouse CD8+ T Cell Isolation Cocktail | 19853C.1 | 1 x 0.5 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™ Streptavidin RapidSpheres™ 50001 | 50001 | 2 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in PBS. |
| 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, 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

Disrupt spleen in PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27260). Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10⁸ 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% FBS and 1 mM EDTA. HBSS, Modified (Without Ca⁺⁺ and Mg⁺⁺; Catalog #37250) can be used in place of PBS. Medium should be free of Ca⁺⁺, Mg⁺⁺, and biotin.

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse CD8+ T Cell Isolation 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 0.25 - 2 mL | 1 x 10 ⁸ cells/mL 0.5 - 8 mL |
| 2 | Add FcR blocker to sample. | 20 µL/mL of sample | 20 µL/mL of sample |
| 3 | Add sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) |
| 4 | Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 10 minutes | RT for 10 minutes |
| 5 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| 6 | Add RapidSpheres™ to sample. | 125 µL/mL of sample | 125 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 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 | <ul style="list-style-type: none"> • Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 2.5 minutes | RT for 2.5 minutes |
| 8 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new 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.

Table 2. EasySep™ Mouse CD8+ T Cell Isolation Kit Protocol

| STEP | INSTRUCTIONS | EASYSEP™ MAGNETS | | |
|------|---|---|---|--|
| | |  EasyPlate™ (Catalog #18102) |  EasyEights™ (Catalog #18103) 5 mL tube |  14 mL tube |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10 ⁸ cells/mL 0.05 - 0.2 mL | 1 x 10 ⁸ cells/mL 0.25 - 2 mL | 1 x 10 ⁸ cells/mL 0.5 - 8 mL |
| 2 | Add FcR blocker to sample. | 20 µL/mL of sample | 20 µL/mL of sample | 20 µL/mL of sample |
| 3 | Add sample to required tube (or plate if using the EasyPlate™ EasySep™ Magnet). | Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018) | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) |
| 4 | Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail. | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 10 minutes | RT for 10 minutes | RT for 10 minutes |
| 5 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds | 30 seconds |
| 6 | Add RapidSpheres™ to sample. | 125 µL/mL of sample | 125 µL/mL of sample | 125 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 5 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 0.25 mL | Top up to 2.5 mL | <ul style="list-style-type: none"> • Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL |
| | Place the tube or plate (without lid) into the magnet and incubate. | RT for 2.5 minutes | RT for 5 minutes | RT for 5 minutes |
| 8 | Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate. | Isolated cells are ready for use | Isolated cells are ready for use | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)

** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Mouse CD8+ T Cell Isolation Kit Protocol

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #21000) |  |
|------|---|---|---|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10 ⁸ cells/mL 0.5 - 6 mL | |
| 2 | Add FcR blocker to sample. | 20 µL/mL of sample | |
| 3 | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) | |
| 4 | Select protocol. | Mouse CD8+ T Cell Isolation 19853 | |
| 5 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | |
| 6 | Load the carousel. | Follow on-screen prompts | |
| | Start the protocol. | Press the green "Run" button | |
| 7 | Unload the carousel when the run is complete. | Isolated cells are ready for use | |

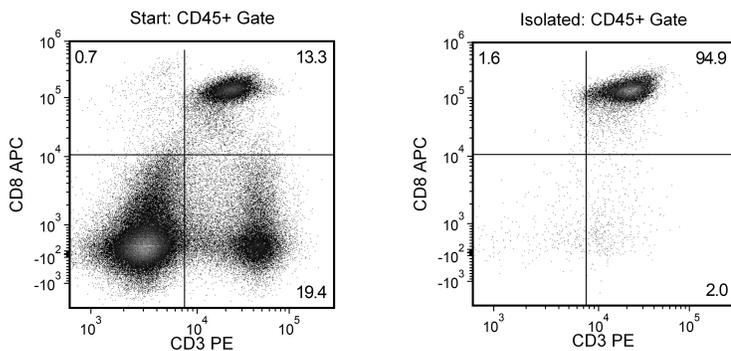
Notes and Tips

ASSESSING PURITY

For purity assessment of CD8+ T cells by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Mouse CD8a Antibody, Clone 53-6.7 (Catalog #60023)

Data



Starting with mouse splenocytes, the CD8+ T cell content (CD3+CD8+) of the isolated fraction is typically 94.4 ± 0.7% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 13.3% and 94.9%, respectively.

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