



EasySep™ Human "Do-It-Yourself" Selection Kit

Positive Selection

Catalog #18099

For processing 1 x 10⁹ cells



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Document #28864 | Version 2_2_0

Description

Isolate highly purified cell types of interest with your own mouse IgG1 monoclonal antibody from human single-cell suspensions by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets any cell type of interest with your own mouse IgG1 monoclonal antibody. 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™ "Do-It-Yourself" Component A | 18090 | 1 x 0.1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ "Do-It-Yourself" Component B | 18091 | 1 x 0.1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ "Do-It-Yourself" Component C | 18092C | 1 x 0.1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody. |
| 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. |
| RoboSep™ Vial for "Do-It-Yourself" Antibody Cocktail | 18093 | 1 vial | Not applicable | Not applicable | Not applicable |

PBS - phosphate-buffered saline

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

Additional Reagent Stability Information

| REAGENT NAME | STORAGE | SHELF LIFE |
|--|----------------------------------|---|
| Selection Cocktail (your own mouse IgG1 monoclonal antibody + Component A + Component B + Component C) | Store at 2 - 8°C. Do not freeze. | Stability should be experimentally determined. Do not exceed the shelf life of the individual components. |

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Prepare a peripheral blood mononuclear cell (PBMC) suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube. If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter clumpy suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 1 x 10⁸ cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

OTHER SAMPLE SOURCES

If other sources or tissues are used, ensure that the cells are in a single-cell suspension.


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.

Table 1. EasySep™ Human "Do-It-Yourself" Selection Kit Protocol

| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) |
|---|---|--|
| 1 | Add your own mouse IgG1 monoclonal antibody (reconstituted if necessary) to a 1.5 mL polypropylene tube. | 15 µg NOTE: Up to 700 µL |
| 2 | Add Component A to the polypropylene tube and mix well. | 100 µL |
| 3 | Add Component B to the polypropylene tube. | 100 µL |
| | Mix and incubate. | 37°C for 5 hours or overnight |
| 4 | Add Component C to the polypropylene tube and mix well. | 100 µL |
| 5 | Add sterile PBS to top up the polypropylene tube to the indicated volume. | Top up to 1 mL |
| Prepared Selection Cocktail is ready to use. | | --- |
| 6 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10 ⁸ cells/mL 0.1 - 2.5 mL NOTE: If starting with fewer than 1 x 10 ⁷ cells, resuspend cells in 0.1 mL For samples with a starting frequency of desired cells < 10%, start with a concentration of 2 x 10 ⁸ cells/mL |
| | Add sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058) |
| 7 | Add Selection Cocktail to sample. | 100 µL/mL of sample |
| | Mix and incubate. | RT for 15 minutes |
| 8 | Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed. | Pipette up and down more than 5 times |
| 9 | Add Magnetic Particles to sample. | 50 µL/mL of sample |
| | Mix and incubate. | RT for 10 minutes |
| 10 | 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 |
| | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes* |
| 11 | 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 |
| 12 | Repeat steps as indicated. | Steps 10 and 11, two more times (total of 3 x 5-minute separations) |
| OPTIONAL ADDITIONAL SEPARATION(S) For samples with a starting frequency of desired cell < 10% NOTE: This will improve purity but may reduce recovery | | Repeat steps 10 and 11, up to three more times (total of 4 - 6 x 5-minute separations) |
| 13 | Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube. | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)


* Recovery may be improved by increasing separation time in the magnet to 10 minutes for each round.

** 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™ Human "Do-It-Yourself" Selection Kit Protocol

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000) |  |
|--|---|---|---|
| 1 | Add your own mouse IgG1 monoclonal antibody (reconstituted if necessary) to the RoboSep™ Vial for "Do-It-Yourself" Antibody Cocktail provided. | 15 µg NOTE: Up to 700 µL | |
| 2 | Add Component A to the RoboSep™ Vial and mix well. | 100 µL | |
| 3 | Add Component B to the RoboSep™ Vial. | 100 µL | |
| | Mix and incubate. | 37°C for 5 hours or overnight | |
| 4 | Add Component C to the RoboSep™ Vial and mix well. | 100 µL | |
| 5 | Add sterile PBS to top up the RoboSep™ Vial to the indicated volume. | Top up to 1 mL | |
| Prepared Selection Cocktail is ready to use. | | --- | |
| 6 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10 ⁸ cells/mL 0.25 - 8.5 mL NOTE: For samples with a starting frequency of desired cells < 10%, start with a concentration of 2 x 10 ⁸ cells/mL | |
| | Add sample to required tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057) | |
| 7 | Select protocol. | Human Do-It-Yourself Positive Selection 18099-high purity | |
| 8 | Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed. | Pipette up and down more than 5 times | |
| 9 | Load the carousel. | Follow on-screen prompts | |
| | Start the protocol. | Press the green "Run" button | |
| 10 | 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 mouse IgG1 antibody used in the cocktail created with the EasySep™ "Do-It-Yourself" Selection Kit may block other fluorochrome-conjugated antibodies that are used to assess purity by flow cytometry. Use fluorochrome-conjugated antibody clones that are not blocked by the antibody clone used in the Selection Cocktail. If this is not possible, one of the following methods can be used to assess purity:

- Use alternative fluorochrome-conjugated markers, if applicable.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

ANTIBODY QUALITY

Recovery of positively selected cells is dependent on the quality of the mouse IgG1 antibody used in the cocktail created with the EasySep™ "Do-It-Yourself" Selection Kit. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

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