

EasySep™ Human B Cell Isolation Kit

For processing 1 x 10¹⁰ cells using the Easy 250 EasySep™ Magnet

Catalog #100-0971

Negative Selection

Document #10000013629 | Version 02



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Description

Isolate untouched and highly purified B cells from fresh leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 99% purity with high recovery
- Untouched, viable cells

This kit targets non-B cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply pipetted off into a new flask. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: This is the Product Information Sheet (PIS) for isolating B cells using the Easy 250 EasySep™ Magnet (Catalog #100-0821). If using other magnets, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

NOTE: Large-format kits are designed for use with large-volume samples. Repeated use with small-volume samples compromises the stability of cocktails and particles, leading to sub-optimal performance, even within the shelf life of the product.

Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|--|-------------|-----------|-------------------------------------|--|---|
| EasySep™ Human B Cell Isolation Cocktail | 300-0510 | 1 x 10 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ Isolation Cocktail Enhancer | 300-0511 | 1 x 10 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A solution that enhances the performance of the isolation cocktail. |
| EasySep™ Dextran RapidSpheres™ | 300-0380 | 1 x 10 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water. |

PBS - phosphate-buffered saline

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

Sample Preparation

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

NOTE: Working with fresh lysed leukapheresis samples is recommended for optimal performance. Alternatively, washed leukapheresis samples may be used for faster sample processing (see below), but a reduction in performance may be observed.

LYSED LEUKAPHERESIS

1. Concentrate the Leukopak (e.g. Human Peripheral Blood Leukopak, Fresh, Catalog #70500*) by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with the recommended medium (e.g. for 200 mL of cells, resuspend in 20 mL of recommended medium).
2. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1-part leukapheresis sample (e.g. for 20 mL of concentrated cells, add 80 mL of Ammonium Chloride Solution).
3. Incubate on ice for 15 minutes.
4. Wash the cells by topping up the tube with the recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
5. OPTIONAL (FOR PLATELET REMOVAL):
 - a. Wash the cells by topping up the tube with the recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
 - b. Repeat step 5a one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 5 x 10⁷ cells/mL in the recommended medium.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

WASHED LEUKAPHERESIS

Wash the fresh peripheral blood leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh) by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 5 x 10⁷ cells/mL in the 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™ Protocol

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

Table 1. EasySep™ Human B Cell Isolation Kit Protocol

| | | EASYSEP™ MAGNETS |
|------|---|--|
| STEP | INSTRUCTIONS | <p>Easy 250 EasySep™ Magnet (Catalog #100-0821)</p>  |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 45 - 225 mL |
| | Add sample to required flask. | T-75 cm ² cell culture flask (i.e. Catalog #200-0500) |
| 2 | Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail. | 50 µL/mL of sample |
| 3 | Add Cocktail Enhancer to sample. NOTE: Do not vortex cocktail. | 50 µL/mL of sample |
| | Mix well and incubate (see Notes and Tips). | RT for 5 minutes |
| 4 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 5 | Add RapidSpheres™ to sample and mix well (see Notes and Tips). | 25 µL/mL of sample |
| | Incubate. | RT for 3 minutes |
| 6 | Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. | <ul style="list-style-type: none"> • Top up to 100 mL for samples ≤ 80 mL • Top up to 250 mL for samples > 80 mL |
| | Place the flask (without cap) into the magnet and incubate. | RT for 10 minutes |
| 7 | Carefully pipette (do not pour) the enriched cell suspension into a new flask. | Use a new T-75 cm ² flask |
| 8 | Remove the flask from the magnet; place the new flask from step 7 (without cap) into the magnet and incubate for a second separation. | RT for 10 minutes |
| 9 | Carefully pipette* (do not pour) the enriched cell suspension into a new tube or centrifuge bottle.* | Use a new tube or centrifuge bottle |
| 10 | Centrifuge sample; carefully aspirate and discard supernatant (see Notes and Tips). | Centrifuge at 300 x g for 10 minutes at RT with low brake |
| | Resuspend to the desired cell concentration using recommended medium. | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)

* e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Notes and Tips

- After the addition of Cocktail, Cocktail Enhancer, and RapidSpheres™, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).
NOTE: Mixing can also be performed by rotating or gently agitating the flask. Cap the flask first to prevent spillage.
- To remove the supernatant, gently sweep the pipette back and forth along the midline of the T-75 cm² flask while aspirating. Avoid touching the sides of the flask. Switch to a 10 mL or smaller serological pipette to collect the residual supernatant.

ASSESSING PURITY

For purity assessment of B cells (CD3-CD19+) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005)
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011; optional)
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional)

Data

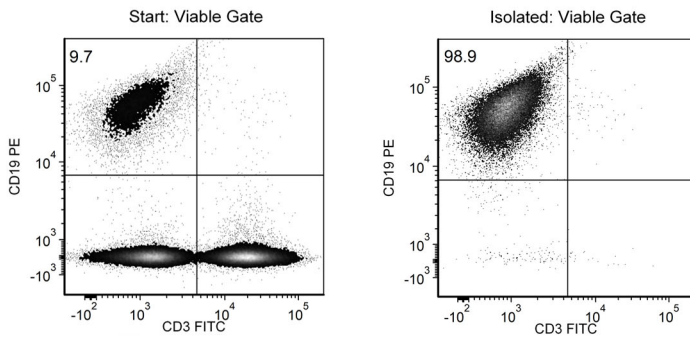


Figure 1. Typical EasySep™ Human B Cell Isolation Profile Using the Easy 250 EasySep™ Magnet

Starting with washed or lysed leukapheresis samples, the B cell content (CD3-CD19+) of the isolated fraction is typically $99.4 \pm 0.5\%$ (gated on viable cells, mean \pm SD for the Easy 250 EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 9.7% and 98.9%, respectively.

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