

EasySep™ Human Monocyte Isolation Kit



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Catalog #100-0697

Negative Selection

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Description

Isolate untouched and highly purified CD14+CD16- monocytes from leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 90% purity with high recovery
- Untouched, viable cells

This kit targets non-monocytes and CD16+ monocytes 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 CD14+CD16- monocytes 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.

Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|--|-------------|-----------|-------------------------------------|--|---|
| EasySep™ Human Monocyte Isolation Cocktail | 300-0360 | 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. Includes an Fc receptor blocking antibody. |
| EasySep™ D Magnetic Particles | 300-0393 | 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 TBS. |

PBS - phosphate-buffered saline; TBS - TRIS-buffered saline

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

Sample Preparation

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

LYSED LEUKAPHERESIS

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the Leukopak.
NOTE: If working with large volumes (> 150 mL), concentrate the Leukopak first 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 recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium and add 30 mL of Ammonium Chloride Solution). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 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 recommended medium.

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

WASHED LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample 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 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.

Table 1. EasySep™ Human Monocyte Isolation Kit Protocol

| STEP | INSTRUCTIONS | Easy 250 EasySep™ Magnet (Catalog #100-0821) |
|------|---|---|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 40 - 225 mL |
| | Add sample to required flask. | T-75 cm ² cell culture flask (i.e. Corning Catalog #353135) |
| 2 | Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail. | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes |
| 3 | Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Add Magnetic Particles to sample. | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes |
| 5 | 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 |
| 6 | Carefully pipette (do not pour) the enriched cell suspension into a new tube or centrifuge bottle. | Use a new tube or centrifuge bottle* |
| 7 | Centrifuge sample; carefully aspirate and discard supernatant. | 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)

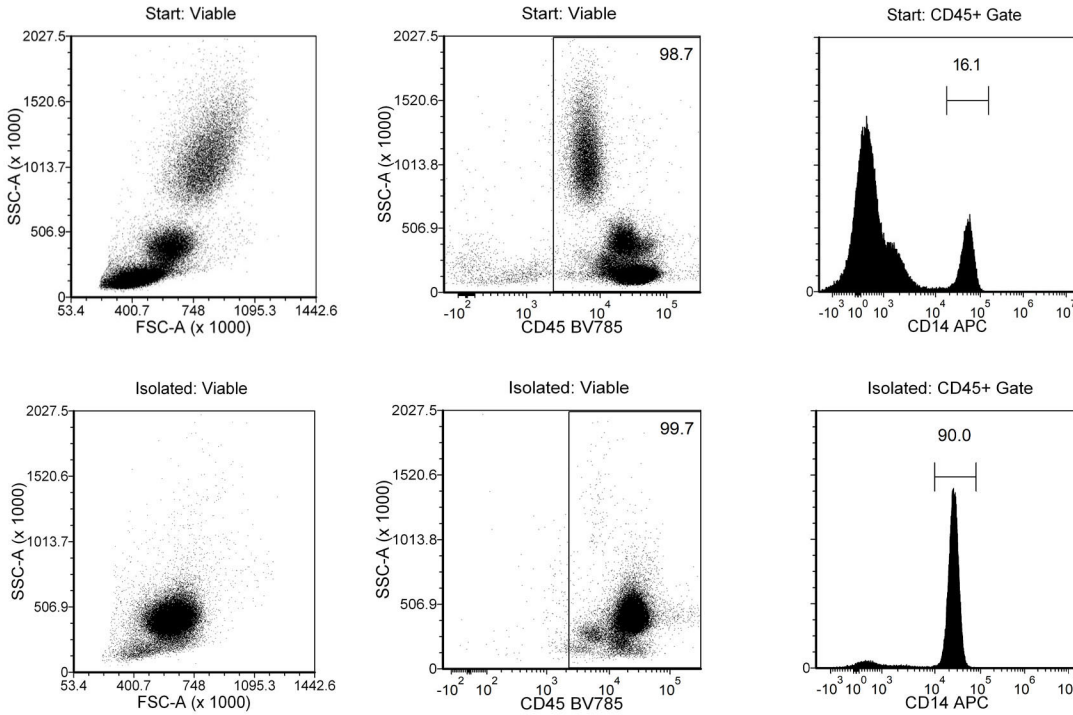
Notes and Tips

ASSESSING PURITY

For purity assessment of monocytes (CD14+CD45+) by flow cytometry, use one of the following fluorochrome-conjugated antibodies:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)
- After addition of Cocktail and Magnetic Particles, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).
NOTE: Mixing can also be done 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.

Data



Starting with washed or lysed leukapheresis samples, the monocyte cell content (CD14+CD45+) of the isolated fraction is typically $88.3 \pm 4.0\%$ (gated on CD45, mean \pm SD for the Easy 250 EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 16.1% and 90.0%, respectively.

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