

EasySep™ Human CD34 Positive Selection Kit II

For processing 1 - 5 x 10⁹ cells

Catalog #17856
#17856RF RoboSep™

Positive Selection

Document #1000000695 | Version 04



Scientists Helping Scientists™ | WWW.STEMCELL.COM

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Description

Isolate highly purified CD34+ cells from fresh or previously frozen mobilized human peripheral blood or bone marrow mononuclear cells (MNCs), previously frozen cord blood MNCs, or human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) cultures by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required

This kit targets CD34+ cells for positive selection with an antibody recognizing the CD34 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.

- If isolating CD34+ cells from fresh cord blood, use EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896)

Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|-------------|----------|-------------------------------------|--|---|
| EasySep™ Human CD34 Positive Selection Cocktail | 17856C | 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. Includes an Fc receptor blocking antibody. |
| EasySep™ Dextran RapidSpheres™ 50100 | 50100 | 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. |

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 and frozen samples, see www.stemcell.com/primarycells.

CELL CONCENTRATION

An initial concentration of 1 - 2 x 10⁸ cells/mL is recommended for primary cells. Depending on the source and condition of the starting cell samples, both primary and cultured cells can sometimes be concentrated up to 5 x 10⁸ cells/mL prior to isolation. In general, higher cell concentrations tend to result in higher purity but lower recovery, whereas lower cell concentrations may improve recovery at the expense of purity.

PERIPHERAL BLOOD or BONE MARROW

Prepare an MNC suspension from whole mobilized peripheral blood or whole bone marrow by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid MNC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube. Older samples may require a longer centrifugation over the density gradient medium in order to reduce contamination by low-density granulocytes. Alternatively, RosetteSep™ Human Granulocyte Depletion Cocktail (Catalog #15624) can be used to deplete total granulocytes before beginning the EasySep™ protocol.

If using previously frozen MNCs, incubate 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 aggregated suspensions through a 37 µm Reversible Cell Strainer (Catalog #27215) for optimal results.

After preparation, resuspend cells in recommended medium.

* SepMate™ (IVD) is available as an in vitro diagnostic (IVD) device in certain regions with an intended use of isolating mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. SepMate™ is manufactured under a cGMP quality management system compliant with 21 CFR 820. In all other regions, SepMate™ is available for research use only (RUO).

SAMPLES WITH HIGH CD34+ CONTENT (> 5%)

Contact us at techsupport@stemcell.com for protocols to further isolate CD34+ cells from samples that have been previously enriched for CD34+ cells.

ESC or iPSC CULTURES

NOTE: If using STEMdiff™ kits, refer to the applicable Technical Manual, available at www.stemcell.com, or contact us to request a copy.

From differentiation cultures containing embryoid bodies (EBs):

1. Transfer the EBs to a 15 mL conical tube (e.g. Catalog #38009) and allow to settle at the bottom of the tube.
2. Remove the medium and add 1 mL of ACCUTASE™ (Catalog #07920). Incubate at 37°C for 10 minutes.
3. Gently pipette up and down 15 times using a 1000 µL pipette tip.
4. Add 10 mL of Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905) and centrifuge.
5. Discard the supernatant, and resuspend the cell pellet in recommended medium.

From differentiation protocols using co-culture on adherent stromal layers:

1. Remove the non-adherent cells and transfer to a 15 mL conical tube.
2. Add 1 mL of ACCUTASE™ to the plate. Incubate at 37°C for 10 minutes.
3. Remove the dissociated cells from the plate by pipetting and transfer to the same 15 mL conical tube.
4. Wash the plate with 10 mL of Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905) and transfer to the same tube.
5. Centrifuge, discard the supernatant, and resuspend the pellet in recommended medium.



Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum and 1 mM EDTA. Medium should be free of Ca⁺⁺ and Mg⁺⁺.

Directions for Use – Manual EasySep™ Protocols

See pages 1 and 2 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™ Human CD34 Positive Selection Kit II Protocol for PERIPHERAL BLOOD, BONE MARROW, or CORD BLOOD


| | | EASYSEP™ MAGNETS | |
|------|--|---|--|
| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) |  “The Big Easy” (Catalog #18001) |
| 1 | Prepare sample as indicated in the recommended medium. | <ul style="list-style-type: none"> If starting with $\leq 1 \times 10^7$ cells, resuspend cells in 0.1 mL If starting with $> 1 \times 10^7$, resuspend cells at $1 - 5 \times 10^8$ cells/mL | <ul style="list-style-type: none"> If starting with $\leq 5 \times 10^7$ cells, resuspend cells in 0.25 mL If starting with $> 5 \times 10^7$ cells, resuspend cells at $1 - 5 \times 10^8$ cells/mL |
| | 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) |
| 2 | Add Selection Cocktail to sample. NOTE: Do not vortex cocktail. | 100 μ L/mL of sample | 100 μ L/mL of sample |
| | Mix and incubate. | RT for 10 minutes | RT for 10 minutes |
| 3 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| 4 | Add RapidSpheres™ to sample.** | 75 μ L/mL of sample | 75 μ L/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 5 | 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 3 mL for samples < 1 mL Top up to 10 mL for samples ≥ 1 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 3 minutes | RT for 3 minutes |
| 6 | 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 |
| 7 | Repeat steps as indicated.** | Steps 5 and 6, four more times (total of 5 x 3-minute separations) | Steps 5 and 6, three more times (total of 4 x 3-minute separations) |
| 8 | 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.

** To improve recovery, addition of particles can be increased to 100 μ L/mL (step 4) and/or the magnetic separation can be reduced by 1 separation to either 4 x 3-minute or 3 x 3-minute separations, depending on the magnet used (step 7). To improve purity, an additional round of magnetic separation may be performed. Note that this additional separation will reduce the recovery.

Table 2. EasySep™ CD34 Positive Selection Kit II Protocol for hESC or hiPSC CULTURES

| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) |
|--|---|--|
| 1 | Prepare sample as indicated in the recommended medium. | <ul style="list-style-type: none"> • If starting with $< 1 \times 10^7$ cells, resuspend cells in 0.1 mL • If starting with $1 \times 10^7 - 1 \times 10^8$ cells, resuspend cells at 1×10^8 cells/mL • If starting with $1 - 5 \times 10^8$ cells, resuspend in 1 mL |
| | Add sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) |
| 2 | Add Selection Cocktail to sample. NOTE: Do not vortex cocktail. | 100 μ L/mL of sample |
| | Mix and incubate. | RT for 10 minutes |
| 3 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Add RapidSpheres™ to sample.* | 50 μ L/mL of sample |
| | Mix and incubate. | RT for 5 minutes |
| 5 | 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 3 minutes |
| 6 | 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 |
| 7 | Repeat steps as indicated. | Steps 5 and 6, two more times (total of 3 x 3-minute separations) |
| OPTIONAL ADDITIONAL SEPARATION NOTE: Purity will increase but recovery may decrease. | | Repeat steps 5 and 6 (total of 4 x 3-minute separations) |
| 8 | 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)


* CD34+ cell recovery can be improved by adding RapidSpheres™ at 100 μ L/mL of cells.

** 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 pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Human CD34 Positive Selection Kit II Protocol for PERIPHERAL BLOOD, BONE MARROW, or CORD BLOOD

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #21000)  |
|------|---|--|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | <ul style="list-style-type: none"> If starting with $< 5 \times 10^7$ cells, resuspend cells in 0.25 mL If starting with $5 \times 10^7 - 2 \times 10^8$ cells, resuspend cells at 2×10^8 cells/mL If starting with $2 - 5 \times 10^8$ cells, resuspend cells in 1 mL If starting with $5 \times 10^8 - 2 \times 10^9$ cells, resuspend cells at 5×10^8 cells/mL |
| | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) |
| 2 | Select protocol. | <ul style="list-style-type: none"> Human CD34 Positive Selection II 17856-high purity Human CD34 Positive Selection II 17856-high recovery |
| 3 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Load the carousel. | Follow on-screen prompts |
| | Start the protocol. | Press the green “Run” button |
| 5 | 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

EasySep™ Human CD34 Positive Selection Cocktail uses a class II anti-CD34 antibody clone that may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. For purity assessment of CD34+ cells by flow cytometry, use one of the following class III fluorochrome-conjugated antibody clones:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), Clone 8G12 (Catalog #60121), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: Flow cytometry analysis of the positively selected cells may show slightly increased side scatter relative to the start sample.

Data

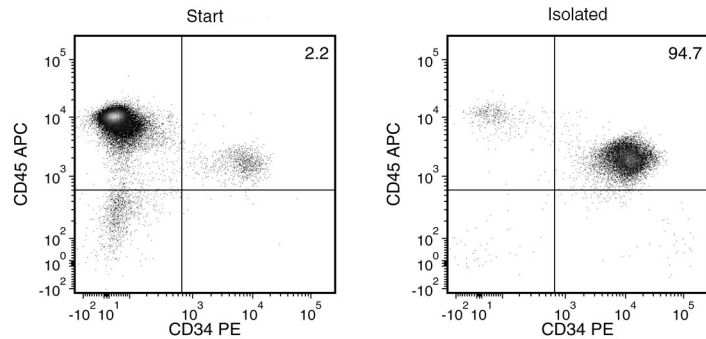


Figure 1. Typical EasySep™ Human CD34 Positive Selection Kit II Isolation Profile

Starting with cord blood, mobilized peripheral blood or bone marrow MNCs, or ESC and iPSC cultures, the CD34+ cell content of the isolated fraction is typically $93.5 \pm 1.1\%$ (mean \pm SD using the purple EasySep™ Magnet). In the above example using frozen cord blood, the purities of the start and final isolated fractions are 2.2% and 94.7%, respectively.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO WWW.STEMCELL.COM/COMPLIANCE.

Copyright © 2025 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, RapidSpheres, RoboSep, RosetteSep, and SepMate are trademarks of STEMCELL Technologies Inc. ACCUTASE is a trademark of Innovative Cell Technologies Inc. Lymphoprep is a trademark of Serumwerk Bernburg AG. The products sold under the Lymphoprep brand name are also manufactured by Serumwerk Bernburg AG. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.