EasySep™ Human CD34 Positive Selection Kit II

For processing 5 x 10^9 cells

Catalog #17856
#17856RF RoboSep™

Positive Selection

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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 (ES) and induced pluripotent stem (iPS) cell 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.

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 hypodense 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 only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of MNCs from whole blood or bone marrow by density gradient centrifugation. 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.

ES or iPS CELL 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 at the indicated cell concentration within the volume range.**	• If starting with < 1 x 10^7 cells, resuspend cells in 0.1 mL • If starting with 1 x 10^7 - 1 x 10^8 cells, resuspend cells at 1 x 10^8 cells/mL • If starting with 1 - 5 x 10^8 cells, resuspend cells in 1 mL	• If starting with < 5 x 10^7 cells, resuspend cells in 0.25 mL • If starting with 5 x 10^7 - 2 x 10^8 cells, resuspend cells at 2 x 10^8 cells/mL • If starting with 2 - 5 x 10^8 cells, resuspend cells in 1 mL • If starting with 5 x 10^8 - 2 x 10^9 cells, resuspend cells at 5 x 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 µL/mL of sample	100 µL/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 µL/mL of sample	75 µL/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	• 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 µL/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 ES or iPS CELL CULTURES

STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**
1	**Prepare sample at the indicated cell concentration using recommended medium.**	• If starting with < 1 x 10^7 cells, resuspend cells in 0.1 mL • If starting with 1 x 10^7 - 1 x 10^8 cells, resuspend cells at 1 x 10^8 cells/mL • If starting with 1 - 5 x 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 µL/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 µL/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 µL/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.**	• If starting with < 5 x 10^7 cells, resuspend cells in 0.25 mL • If starting with 5 x 10^7 - 2 x 10^8 cells, resuspend cells at 2 x 10^8 cells/mL • If starting with 2 - 5 x 10^8 cells, resuspend cells in 1 mL • If starting with 5 x 10^8 - 2 x 10^9 cells, resuspend cells at 5 x 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.**	• 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

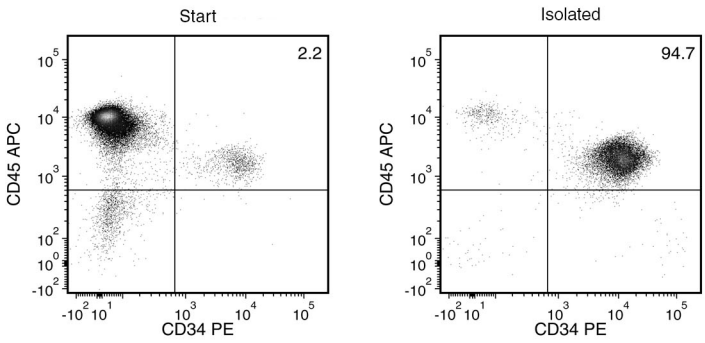
ASSESSING PURITY

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



Starting with cord blood, mobilized peripheral blood or bone marrow MNCs, or ES and iPS cell cultures, the CD34+ cell content of the isolated fraction is typically 93.5 ± 1.1% (mean ± 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.