EasySep™

Positive Selection

Catalog #18167

EasySep™ hESC-Derived CD34 Positive Selection Kit

For processing 5 x 10^9 cells

STEMCELL™ TECHNOLOGIES

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Document #29923 | Version 1_0_4

Description

Isolate highly purified CD34+ cells from 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 Selection Kit II (Catalog #17896).
- If isolating CD34+ cells from fresh whole blood or buffy coat, use EasySep™ Whole Blood CD34 Selection Kit (Catalog #18086).
- If isolating CD34+ cells from any other sample type, use EasySep™ Human CD34 Positive Selection Kit (Catalog #18056).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ hESC-Derived CD34 Positive Selection Cocktail	18167C	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™ 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.

PBS - phosphate-buffered saline

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

Sample Preparation

ES or iPS CELL CULTURES

From differentiation cultures containing embryoid bodies (EBs):

1. Transfer the EBs to a 15 mL conical tube and allow to sediment.
2. Remove the medium and add 1 mL of ACCUTASE™ (Catalog #07920).
3. Incubate for 10 minutes at 37°C, then gently pipette up and down 15 times using a P1000.
4. Add 10 mL of Dulbecco's PBS (D-PBS) with 2% Fetal Bovine Serum (FBS; 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 and 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 D-PBS with 2% FBS and transfer to the same tube.
5. Centrifuge, discard the supernatant, and resuspend the pellet in recommended medium.

If aggregates remain, 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. Ensure the buffer used with DNase I Solution and cell suspension is free of EDTA. Filter aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at the concentration indicated in Table 1 in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), 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™ hESC-Derived CD34 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**
1	**Prepare sample at the indicated cell concentration using recommended medium.**	• If starting with < 1 x 10^7 total cells, resuspend cells in 0.1 mL • If starting with 1 x 10^7 - 1 x 10^8 total cells, resuspend cells at 1 x 10^8 cells/mL • If starting with 2 - 5 x 10^8 total 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 Positive Selection Cocktail to sample.**	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
4	**Add Magnetic Particles to sample.***	50 µL/mL of sample
	Mix and incubate.	RT for 10 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 10 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 10-minute separations)
OPTIONAL ADDITIONAL SEPARATION **NOTE: Purity will increase but recovery may decrease.**		Steps 5 and 6 (total of 4 x 10-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 Magnetic Nanoparticles at a concentration of 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.

Notes and Tips

ASSESSING PURITY

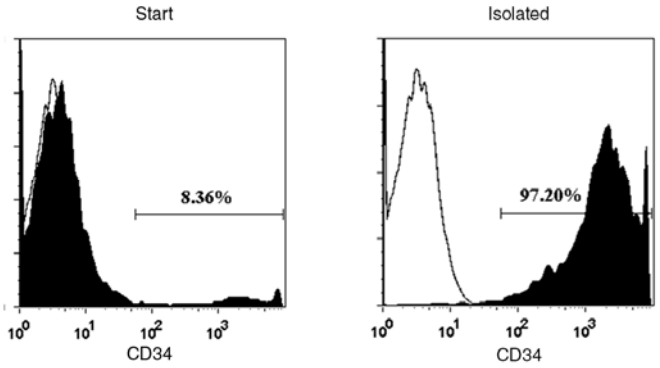
The EasySep™ Human CD34 Positive Selection Cocktail uses a class II anti-CD34 antibody clone and may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. Alternate clones for CD34 positive selection are available as custom kits. Contact us at techsupport@stemcell.com for more information.

For purity assessment of CD34+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121), clone AC136, or clone Birma K3

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

Data



Starting with a differentiated population containing at least 5% CD34+ cells, the CD34+ cell content of the isolated fraction typically ranges from 84 - 99%. In the above example, the purities of the start and final isolated fractions are 8.36% and 97.20%, respectively.

Data courtesy of D. Kaufman, University of Minnesota.