EasySep™ Human CXCR4 Positive Selection Kit

Positive Selection

Catalog #18163

For processing 1 x 10^9 cells

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Description

Isolate highly purified CXCR4+ cells derived from human pluripotent stem cells by immunomagnetic positive selection.

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

This kit targets CXCR4+ cells for positive selection with an antibody recognizing the CXCR4 (CD184) surface marker. The cocktail contains the B-R24 clone of anti-CXCR4 antibody which is known to inhibit HIV infection. 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CXCR4 Positive Selecton Cocktail	18163C	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.
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) and should be stored as indicated above.

Sample Preparation

Trypsin-EDTA is recommended for preparation of a single-cell suspension from human pluripotent stem cell-derived definitive endoderm.

1. Discard culture medium and rinse each well of a 6-well plate with 2 mL of DMEM/F-12 with 15 mM HEPES (Catalog #36254).
2. Dilute Trypsin-EDTA (0.25%; Catalog #07901) 10-fold in HBSS, Modified (Without Ca++ and Mg++; Catalog #37250).
3. Add 1 mL of diluted Trypsin-EDTA to each well.
4. Incubate at 37°C for 5 minutes until the cells are easily dislodged from the bottom of the plate with gentle tapping.
5. Pipette up and down 3 - 4 times with a 1 mL pipette tip to dissociate to single cells.
6. Combine the single-cell suspension from each well into a 15 mL conical tube (e.g. Catalog #38009).
7. Wash each well with 1 mL of Soybean Trypsin Inhibitor, ACF (Catalog #07457) and add to the 15 mL conical tube.
8. Centrifuge at 300 x g for 5 minutes.
9. Remove supernatant and resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

Recommended Medium

RoboSep™ Buffer 2 (Catalog #20164), or PBS containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use

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

Table 1. EasySep™ Human CXCR4 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**EasySep™ (Catalog #18000)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	5 x 10^7 cells/mL 0.1- 0.3 mL NOTE: If starting with fewer than 5 x 10^6 cells, resuspend cells in 0.1 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.**	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
4	**Add RapidSpheres™ to sample.**	25 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 5 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; the tube contains the isolated cells.**	Discard supernatant
7	**Repeat steps as indicated.**	Steps 5 and 6, two more times (total of 3 x 5-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)

* 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

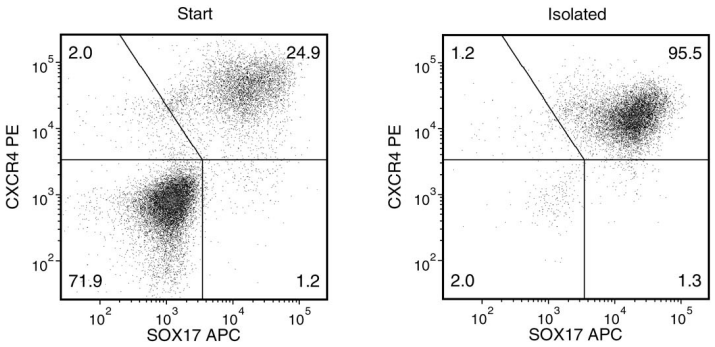
EasySep™ Human CXCR4 Positive Selection Cocktail uses the anti-CXCR4 antibody clone B-R24. For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD184 (CXCR4) Antibody, Clone12G5 (partial blocking; Catalog #60089)
- Anti-Human CD117 (c-Kit) Antibody, Clone 104D2 (Catalog #60087)
- Anti-human SOX17 antibody

The following method can also be used:

- Use an alternative fluorochrome-conjugated antibody marker.

Data



Starting with human pluripotent stem cell-derived definitive endoderm, the CXCR4+SOX17+ cell content of the isolated fraction is typically 92.9 ± 3.1% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 24.9% and 95.5%, respectively.