EasySep™ Human CD271 Selection Kit

Positive Selection

Catalog #18659

For processing 2 x 10^9 cells

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Description

Isolate highly purified CD271+ cells from fresh human bone marrow mononuclear cells (MNCs) by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets CD271+ cells for positive selection with an antibody recognizing the CD271 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD271 Positive Selection Cocktail	18659C	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™ 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.
Anti-Human CD32 (Fc gamma RII) Blocker, for Positive Selection	18520	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.

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.

BONE MARROW

Prepare an MNC suspension from whole bone marrow (BM) by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801).

1. Perform cell count on a fresh BM sample by removing 10 µL of BM and diluting 1 in 100 with 3% Acetic Acid with Methylene Blue (Catalog #07060). Count cells using a hemocytometer.
2. Dilute the BM with recommended medium at room temperature (15 - 25°C) to a total volume of 70 mL (approximately 1 in 2.8; e.g. dilute 25 mL of BM with 45 mL of recommended medium).
3. Add 16 mL of Lymphoprep™ to each of three 50 mL conical tubes. Carefully layer 23.3 mL of diluted BM carefully on top of the Lymphoprep™ in each tube.
4. Centrifuge at 300 x g for 30 minutes at room temperature with the brake off.
5. Remove and discard the upper plasma layer without disturbing the plasma:density gradient medium interface. Carefully remove and retain the MNCs at the interface layer and place into a new 50 mL conical tube.
6. Resuspend the MNCs with 45 mL of cold (2 - 8°C) recommended medium. Centrifuge at 200 x g for 10 minutes.
7. Discard the supernatant and repeat step 6.
8. Discard the supernatant and resuspend at a final concentration of 1 x 10^8 cells/mL in cold recommended medium.

For more rapid MNC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

* 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).

Recommended Medium

PBS containing 2% fetal bovine serum (FBS) and 2 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocol

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

Table 1. EasySep™ Human CD271 Selection Kit Protocol

STEP	INSTRUCTIONS	EasySep™ (Catalog #18000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.5 - 1 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)
2	Add FcR blocker to sample.	25 µL/mL of sample
3	Add Selection Cocktail to sample. *	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes
4	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
5	Add Magnetic Particles to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes
6	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
7	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
8	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 5-minute separations)
OPTIONAL ADDITIONAL SEPARATION NOTE: This will improve purity but may reduce recovery.		Steps 6 and 7 (total of 4 x 5-minute separations)
9	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)
* If starting with tissue samples other than bone marrow, titration of Selection Cocktail is necessary. Titrate cocktail concentrations in the range of 50 - 150 µL/mL.
** 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 CD271 Positive Selection Cocktail uses an anti-CD271 antibody clone that partially blocks other anti-CD271 antibody clones. Using an anti-CD271 antibody to assess purity by flow cytometry will underestimate purity and recovery. The following method can also be used to assess purity:

- Use the colony forming unit - fibroblast (CFU-F) assay (described below) to assess purity of enriched CD271+ cells. Enriched populations should yield a 9- to 60-fold increase in CFU-F colonies compared to unselected density gradient medium-isolated BM samples.

CFU-F ASSAY

Plate duplicates of 3 different cell densities in complete MesenCult™ Medium (Human; Catalog #05411) as follows:

- Plate the pre-enriched MNCs in T-25 cm^2 flasks at 1 x 10^6 cells/flask, 2 x 10^6 cells/flask, and 3 x 10^6 cells/flask. If using tissue culture-treated 6-well flat-bottom plates, use 2 x 10^5 cells/well, 5 x 10^5 cells/well, and 1 x 10^6 cells/well.
- Plate post-enriched CD271-selected bone marrow fraction at 10-fold lower cell densities than above (e.g. plate 1 x 10^5 cells/flask, 2 x 10^5 cells/flask, and 3 x 10^5 cells/flask if using T-25 cm^2 flasks).
- Place the tissue culture flasks/plates into a 37°C humidified incubator with 5% CO_2 in air and > 95% humidity. Count colonies after 14 days of incubation.

NOTE: For more information on how to perform the CFU-F assay, refer to the Technical Manual: Culture of Human Mesenchymal Stem Cells Using MesenCult™-XF Medium (Document #29184).