RosetteSep™ Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail

Catalog #15027	1 x 2 mL	For processing 40 mL bone marrow
Catalog #15067	5 x 2 mL	For processing 200 mL bone marrow

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Description

Enrich untouched bone marrow progenitor cells from human whole bone marrow by negative selection.

- Fast and easy-to-use
- Requires no special equipment or training
- Untouched, viable cells

This kit targets non-progenitor cells for removal with antibodies recognizing specific cell surface markers. The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes. This increases the density of the unwanted (rosetted) cells, such that they pellet along with the free RBCs when centrifuged over a density gradient medium. Desired cells are never labeled with antibody and are easily collected as a highly enriched population at the interface between the plasma and the density gradient medium. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail	15027C	2 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.

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

BONE MARROW

Use an unprocessed bone marrow sample.

Although RosetteSep™ has been optimized for use with whole blood or bone marrow, cells can be enriched from other sources (i.e. buffy coat) provided that RBCs are present at a ratio of at least 100 RBCs per nucleated cell. The concentration of nucleated cells in the sample should not exceed 5 x 10^7 cells/mL.

Recommended Medium

Dulbecco's PBS containing 2% fetal bovine serum (Catalog #07905).

Density Gradient Medium

Lymphoprep™ (Catalog #07801) or other density gradient medium with a density of 1.077 g/mL.

Directions for Use – RosetteSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium.

Ensure that bone marrow sample, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C).

Table 1. RosetteSep™ Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail Protocol

STEP	INSTRUCTIONS	ROSETTESEP™
1	Collect sample.	Up to 15 mL per tube (see Table 2)
2	Add RosetteSep™ Cocktail to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 20 minutes
3	Dilute sample with recommended medium and mix gently.	Equal volume to sample
4	Add density gradient medium to required tube.	See Table 2 for volumes and tubes
5	Add diluted sample to the tube containing the density gradient medium.	Layer diluted sample on density gradient medium, being careful to minimize their mixing
6	Centrifuge.	1200 x g for 20 minutes, **brake off**
7	Collect enriched cells. * For platelet removal see footnote below.	Harvest enriched cell layer with a pipette and transfer to new tube**
8	Wash enriched cells.	Top up with recommended medium
9	Centrifuge.	300 x g for 10 minutes brake low
		Discard supernatant
10	Repeat steps as indicated.	Steps 8 and 9***
11	Resuspend cells in recommended medium.	The enriched cells are ready for use.

RT - room temperature (15 - 25°C)
* To minimize platelet contamination, remove and discard the top third of the plasma layer before collecting the cells at the density gradient medium:plasma interface. Platelets may also be removed by including an extra wash with centrifugation at 120 x g for 10 minutes at room temperature with no brake after step 9.
** Sometimes it is difficult to see the cells at the interface. It is recommended to remove some of the density gradient medium along with the pre-enriched cells in order to ensure complete recovery.
*** One of the wash steps can be done with Ammonium Chloride Solution (Catalog #07800) prior to flow cytometry analysis or if residual RBCs will interfere with subsequent assays.

Table 2. Recommended Volumes and Tube Sizes

BONE MARROW VOLUME	RECOMMENDED MEDIUM VOLUME	TUBE SIZE	DENSITY GRADIENT MEDIUM VOLUME
0.5 mL	0.5 mL	5 mL	1.5 mL
1 mL	1 mL	5 mL	1.5 mL
2 mL	2 mL	14 mL	3 mL
3 mL	3 mL	14 mL	3 mL
4 mL	4 mL	14 mL	4 mL
5 mL	5 mL	50 mL	15 mL
10 mL	10 mL	50 mL	15 mL
15 mL	15 mL	50 mL	15 mL

* Small bubbles may be present in the density gradient medium after pipetting. This will not affect performance.

Notes and Tips

CONVERSION OF g TO RPM

To convert g to RPM, use the following formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) \times (Radius)}}$$

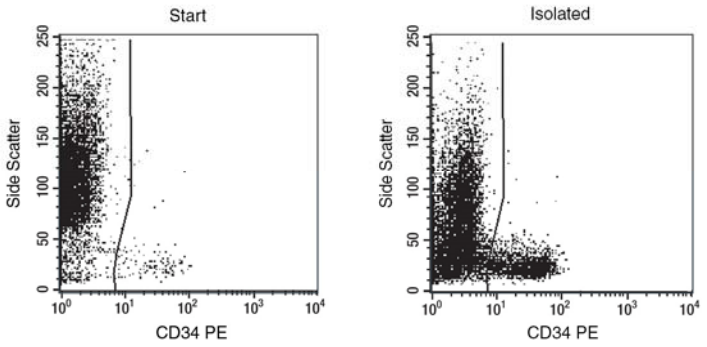
Where: RCF = relative centrifugal force (g)
RPM = centrifuge speed in revolutions per minute
Radius = radius of rotor in cm

ASSESSING PURITY

For purity assessment of bone marrow progenitor cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

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

Data



Starting with whole bone marrow, the bone marrow progenitor cell enrichment of the isolated fraction is typically 25 ± 10-fold. In the above example, the purities of the start and final isolated fractions are 0.6% and 17%, respectively (29-fold enrichment of CD34+ cells).