

**RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Kit**



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Catalog #15226      2 x 2.5 mL      For processing 1000 mL cord blood  
 Catalog #15266      10 x 2.5 mL      For processing 5000 mL cord blood

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## Description

Enrich untouched hematopoietic progenitor cells directly from human cord blood by negative selection.

- Fast and easy-to-use
- Requires no special equipment or training
- Untouched, viable cells
- Can be combined with SepMate™ for consistent, high-throughput sample processing

This kit targets non-hematopoietic progenitor cells for removal with antibodies recognizing specific cell surface markers. The RosetteSep™ antibody cocktail crosslinks unwanted cells in human cord 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 Progenitor Cell Basic Pre-Enrichment Cocktail | 15226C      | 2 x 2.5 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

For available fresh and frozen samples, see [www.stemcell.com/primarycells](http://www.stemcell.com/primarycells).

### CORD BLOOD

For optimal performance use cord blood collected within the last 24 hours and stored at room temperature (15 - 25°C). Remove aggregates by passing cord blood through a 70 µm cell strainer. For more rapid RosetteSep™ processing this product can be combined with the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD\* (Catalog #85450/85415) cell isolation tube. For more information on SepMate™ see the associated Product Information Sheets.

If using SepMate™ with samples with hematocrits outside the normal range, please note that a minimum packed RBC volume is required. See table below for details.

|                           | SEPMATE™ - 15 | SEPMATE™ - 50 |
|---------------------------|---------------|---------------|
| Sample volume range       | 0.5 - 5 mL    | 4 - 17 mL     |
| Minimum packed RBC volume | 0.25 mL       | 2 mL          |
| Maximum packed RBC volume | 3 mL          | 12 mL         |

- For samples with low hematocrits, the required sample volume may be greater than the minimum volume stated above.
- For samples with very high hematocrits, the maximum sample volume may be less than the maximum volume stated above.

\* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (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

Dulbecco's Phosphate Buffered Saline with 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 cord blood sample, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C). For more information on the use of the SepMate™-15 or SepMate™-50 tube, refer to the applicable Product Information Sheet.

**Table 1. RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Kit Protocol**

| STEP | INSTRUCTIONS  | ROSETTESEP™   |   |
|------|---|---|---|
|      |   | Standard Tube   | SepMate™ Tube   |
| 1    | Collect sample.   | Up to 15 mL per tube (see Table 2)  | 0.5 - 17 mL per tube (see Table 2)  |
| 2    | Add RosetteSep™ Cocktail to sample.   | 5 µL/mL of sample   | 5 µL/mL of sample   |
|      | Mix and incubate.   | RT for 10 minutes   | RT for 5 minutes  |
| 3    | Dilute sample with recommended medium and mix gently.                                       | Equal volume to sample  | Equal volume to sample  |
| 4    | Add density gradient medium to required tube.   | See Table 2 for volumes and tubes   | 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 | Pour or pipette diluted sample into tube  |
| 6    | Centrifuge.   | 1200 x g for 20 minutes, <b>brake off</b>   | 1200 x g for 10 minutes, <b>brake on</b><br>NOTE: For samples > 24 hours old it may be necessary to centrifuge for an additional 10 minutes.                              |
| 7    | Collect enriched cells.<br>* For platelet removal see footnote below.                       | Harvest enriched cell layer with a pipette and transfer to new tube**                   | Pour supernatant into a new standard tube<br>NOTE: Some RBCs may be present on the surface of the SepMate™ insert after centrifugation. This will not affect performance. |
| 8    | Wash enriched cells.  | Top up with recommended medium  | Top up with recommended medium  |
| 9    | Centrifuge.   | 300 x g for 8 minutes<br>brake low  | 300 x g for 8 minutes<br>brake low  |
|      |   | Discard supernatant   | Discard supernatant   |
| 10   | Repeat steps as indicated.  | Steps 8 and 9***<br>NOTE: For the second wash, centrifuge at 120 x g for 10 minutes     | Steps 8 and 9***<br>NOTE: For the second wash, centrifuge at 120 x g for 10 minutes   |
| 11   | Resuspend cells in recommended medium containing 1 mM EDTA per 100 mL of cord blood sample. | The enriched cells are ready for use  | 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 after step 9, by centrifugation at 120 x g for 10 minutes at room temperature with no brake.

\*\* 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**

| CORD BLOOD VOLUME | RECOMMENDED MEDIUM VOLUME | STANDARD TUBE |                                | SEPMATE™ TUBE |                                 |
|-------------------|---------------------------|---------------|--------------------------------|---------------|---------------------------------|
|                   |                           | TUBE SIZE     | DENSITY GRADIENT MEDIUM VOLUME | TUBE SIZE     | DENSITY GRADIENT MEDIUM VOLUME* |
| 0.5 mL            | 0.5 mL                    | 5 mL          | 1.5 mL                         | 15 mL         | 4.5 mL                          |
| 1 mL              | 1 mL                      | 5 mL          | 1.5 mL                         | 15 mL         | 4.5 mL                          |
| 2 mL              | 2 mL                      | 14 mL         | 3 mL                           | 15 mL         | 4.5 mL                          |
| 3 mL              | 3 mL                      | 14 mL         | 3 mL                           | 15 mL         | 4.5 mL                          |
| 4 mL              | 4 mL                      | 14 mL         | 4 mL                           | 15 mL / 50 mL | 4.5 mL** / 15 mL                |
| 5 mL              | 5 mL                      | 50 mL         | 15 mL                          | 15 mL / 50 mL | 3.5 mL / 15 mL                  |
| 10 mL             | 10 mL                     | 50 mL         | 15 mL                          | 50 mL         | 15 mL                           |
| 15 mL             | 15 mL                     | 50 mL         | 15 mL                          | 50 mL         | 15 mL                           |
| 17 mL             | 17 mL                     | --            | --                             | 50 mL         | 15 mL                           |

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

\*\* If using a sample size of > 4 - 5 mL in the SepMate™-15 tube, use 3.5 mL of density gradient medium.

## Notes and Tips

### CONVERSION OF g TO RPM

To convert g to RPM, use the following formula:

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

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

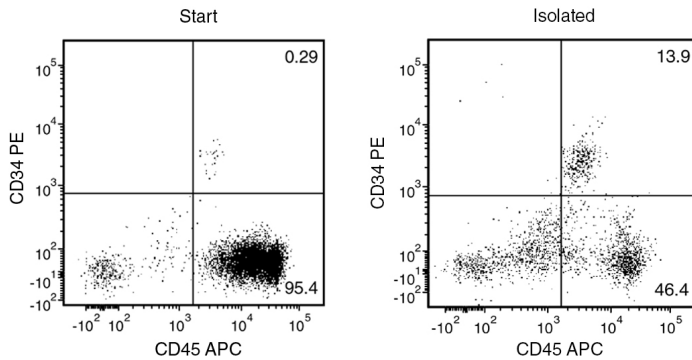
### ASSESSING PURITY

For purity assessment of cord blood progenitor cells by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), or
- Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: Lyse RBCs prior to flow cytometry.

## Data



Starting with fresh human cord blood, the CD34+CD45+ viable cell content of the pre-enriched fraction typically ranges from 2 - 16%. In the above example, the purities of the start and final enriched fractions are 0.3% and 13.9%, respectively.

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