**DIRECTIONS FOR USE**

Ensure that bone marrow sample, phosphate-buffered saline with 2% fetal bovine serum (PBS + 2% FBS; Catalog #07905), density gradient medium (see Notes and Tips, reverse page), and centrifuge are all at room temperature (15 - 25°C).

1. Add RosetteSep™ Enrichment Cocktail at 50 μL/mL of bone marrow (e.g. for 2 mL of bone marrow, add 100 μL of cocktail). Mix well.
   
   **Note:** If using samples other than fresh bone marrow, please see Notes and Tips.

2. Incubate **20 minutes** at room temperature (15 - 25°C).

3. Dilute sample with an equal volume of PBS + 2% FBS and mix gently.

4. Layer the diluted sample on top of the density gradient medium OR Layer the density gradient medium underneath the diluted sample.
   
   **Note:** Be careful to minimize mixing of the density gradient medium and sample.

   See table below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL density gradient medium to make it easier to remove the enriched cell layer.

<table>
<thead>
<tr>
<th>BONE MARROW (mL)</th>
<th>PBS + 2% FBS (mL)</th>
<th>DENSITY GRADIENT MEDIUM (mL)</th>
<th>TUBE SIZE (mL)</th>
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<tr>
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<td>1.5</td>
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<td>2</td>
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<td>50</td>
</tr>
</tbody>
</table>

5. Centrifuge for **20 minutes** at 1200 x g (see Notes and Tips) at room temperature (15 - 25°C), with the brake off.

6. Remove the enriched cells from the density gradient medium:plasma interface.
   
   **Note:** Sometimes it is difficult to see the cells at the interface, especially when very rare cells are enriched. It is advisable to remove some of the density gradient medium along with the enriched cells in order to ensure their complete recovery.

7. Wash enriched cells with PBS + 2% FBS. Repeat.

8. Use enriched cells as desired. We recommend that enriched samples are lysed with Ammonium Chloride Solution (Catalog #07800) to remove residual red blood cells (RBCs) prior to flow cytometric analysis (this can be done as one of the wash steps) or if residual RBCs will interfere with subsequent assays.

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**ROSETTESEP™ PROTOCOL DIAGRAM**

1. Add RosetteSep™ antibody cocktail
2. Incubate **20 minutes** at room temperature (15 - 25°C)
3. Layer over density gradient medium
4. Spin
5. Collect
6. plasma
7. Enriched cells (unrosetted)
8. Desired cells
9. Unwanted cells
10. Red cells

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS. FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.
CATALOG #15129 2 mL For labeling 40 mL of bone marrow
CATALOG #15169 10 mL For labeling 200 mL of bone marrow

PRODUCT DESCRIPTION AND APPLICATIONS:
The RosetteSep™ Human Multiple Myeloma Enrichment Cocktail is designed to enrich myeloma cells (B and plasma cells) from fresh patient bone marrow aspirates.

ROSETTESEP™ LABELING OF HUMAN CELLS
The RosetteSep™ antibody cocktail crosslinks unwanted cells in human bone marrow to multiple RBCs, forming immunorosettes (Figure 1). 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.

NOTES AND TIPS
RECOMMENDED MEDIUM
The recommended medium is PBS + 2% FBS (Catalog #07905).

DENSITY GRADIENT MEDIUM
Density gradient medium refers to Lymphoprep™ (Catalog #07801), Ficoll-Paque™ PLUS, or other similar density gradient media.

CONVERSION of g to RPM
To convert g to rpm, use the following formula:

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

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

SAMPLES OTHER THAN WHOLE BLOOD
Although RosetteSep™ has been optimized for use with whole blood and bone marrow, cells can be enriched from other sources (i.e. buffy coat, leukapheresis). The concentration of nucleated cells in the sample should not exceed 5 x 10^7 cells/mL, and RBCs should be present at a ratio of at least 50 - 100 RBCs per nucleated cell.

ASSESSING PURITY
The purity of enriched cells is dependent on the percentage of myeloma cells in the starting bone marrow sample. Purity is expected to be > 95% when measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD138 antibody such as Anti-Human CD138 (Syndecan-1) Antibody, Clone MI15 (Catalog #60003).

COMPONENT DESCRIPTION:
ROSETTESEP™ HUMAN MULTIPLE MYELOMA ENRICHMENT COCKTAIL  
CODE #15129C
This cocktail contains a combination of monoclonal antibodies. These antibodies are bound in bispecific Tetrameric Antibody Complexes (TACs) which are directed against cell surface antigens on non-myeloma cells. It should be kept in mind that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable. Precipitate may be observed in the cocktail vial but will not affect performance.

STABILITY AND STORAGE:
ROSETTESEP™ HUMAN MULTIPLE MYELOMA ENRICHMENT COCKTAIL
Product stable at 2 - 8°C until expiry date as indicated on label. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

REFERENCES:

Figure 1 Rosette of unwanted cell and RBCs formed by RosetteSep™ Tetrameric Antibody Complexes (TACs)