

Buffy Coat Procedure



Although RosetteSep® has been optimized for use with whole blood, cells can be enriched from other sources, provided that:

- The concentration of nucleated cells does not exceed 5×10^7 cells/mL,
- Red blood cells (RBCs) are present at a ratio of at least 30 RBCs per nucleated cell.

A. Buffy Coat preparation from whole blood

1. Spin whole blood sample at 200 x g for 10 minutes at room temperature with the brake off.
2. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated RBCs.
3. Count the RBCs: dilute a small fraction of the sample with PBS or any other isotonic solution and determine the cell concentration with a hemocytometer. Most of the cells counted in PBS will be RBCs.

Count the nucleated cells: dilute a small fraction of the sample in acetic acid (1:10) to lyse the RBCs and determine the nucleated cell concentration with a hemocytometer.

The ratio of the two counts should be at least 30 RBCs per nucleated cell.

4. Adjust the sample volume to ensure that nucleated cells are not more concentrated than 5×10^7 cells per mL.

Notes:

1. A buffy coat suspension can be collected in tubes or in the blood collection bag (where applicable).
2. A buffy coat suspension is a concentrated leukocyte suspension. It is not mononuclear: the granulocytes are still present.
3. Platelet contamination can be decreased by collecting less of the plasma.

B. RosetteSep® procedure for Buffy Coat

1. Add 50 µL of cocktail per mL of buffy coat suspension. Mix well.

Note: if you are using the cord blood progenitor cocktail, (#15026/15066) add **75 µL** of RosetteSep® cocktail to the buffy coat per 10 mL of original cord blood volume and mix well.

2. Incubate 20 minutes at room temperature.
3. Dilute the sample with **2 times** the volume of PBS + 2% FBS (#07905) and mix gently.
4. Layer the diluted sample on top of Ficoll®.

Be careful to minimize mixing of Ficoll® and sample. See the table for recommended Ficoll® volumes based on tube size and sample volume.

Recommended Volumes and Tube Sizes			
Sample	PBS + 2% FBS	Ficoll®	Tube Size
1 mL	2 mL	1.5 mL	5 mL
2 mL	4 mL	3 mL	14 mL
3 mL	6 mL	4 mL	14 mL
4 mL	8 mL	15 mL	50 mL
5 mL	10 mL	15 mL	50 mL
10 mL	20 mL	15 mL	50 mL

5. Centrifuge for 20 minutes at 1200 x g at room temperature, with the brake off.
6. Remove the enriched cells from the Ficoll®: plasma interface.
7. Wash enriched cells with PBS + 2% FBS.
8. Use enriched cells as desired. We recommend that enriched samples are lysed with ammonium chloride to remove residual red blood cells prior to flow cytometric analysis (this can be done as one of the wash steps) or if residual red blood cells will interfere with subsequent assays.

Please note that RosetteSep® has been designed and tested for isolation from whole blood, bone marrow or cord blood, as indicated in the procedure shipped with the product, and not for buffy coat. The above guidelines are for information purposes only, to assist you in designing your own procedure.

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