Isolating Specific Cell Subsets from Buffy Coat or Leukapheresis Samples Without Columns or Magnets

The following procedure provides guidelines for isolating cells from samples other than whole blood (e.g. buffy coat sample or leukapheresis) using RosetteSep™, an immunodensity cell separation platform that isolates specific cell subsets during density gradient centrifugation. RosetteSep™ first crosslinks unwanted cells to red blood cells (RBCs) present in the sample to form immunorosettes. When the sample is centrifuged over a density gradient medium (e.g. Ficoll™ or Lymphoprep™), the immunorosettes pellet, leaving highly purified cells at the interface between the plasma and the density gradient medium.

In order to perform RosetteSep™ cell enrichment successfully, it is essential that the sample contain sufficient RBCs; we recommend a ratio of RBCs:WBCs of at least 50:1, and preferably 100:1. This is much more than is commonly found in buffy coat samples.

This is a general procedure; specific conditions may vary according to the cell type being enriched. Find RosetteSep™ protocols for a specific cell type by clicking here.

Sample Preparation

Samples should be resuspended at a white blood cell concentration not exceeding 5 x 10^7 cells / mL.

Cell Isolation

1. Ensure that the sample, PBS + 2% FBS (phosphate buffered saline + 2% fetal bovine serum), density gradient medium, and centrifuge are all at room temperature.
2. Add RosetteSep™ cocktail to sample and mix well.
3. Incubate 20 minutes at room temperature.
4. Dilute the sample with **2 times** the volume of PBS + 2% FBS and mix gently.
5. Layer the diluted sample on top of the density gradient medium using the recommended volumes (Table 1). Be careful to minimize mixing of the density gradient medium and the sample.

Table 1. Recommended Volumes and Tube Sizes

Sample Size (mL)	Tube Size (mL)	PBS + 2% FBS (mL)	Density Gradient Medium (mL)
1	5	1	1.5
2	14	2	3
3	14	3	3
4	14	4	4
5	50	5	15
10	50	10	15
15	50	15	15

With 50 mL centrifuge tubes, use a minimum of 15 mL density gradient medium to make it easier to remove the enriched cell layer.

6. Centrifuge for 20 minutes at 1200 x g at room temperature, with the brake off. (G to RPM Converter)
7. Remove the enriched cells from the density gradient medium:plasma interface.Sometimes it is difficult to see cells at the interface, especially when very rare cells are enriched. To maximize recovery in these instances, remove some of the density gradient medium along with the enriched cells.
8. Wash enriched cells with PBS + 2% FBS. Repeat wash.
9. Use enriched cells as desired. We recommend that residual red blood cells are removed from samples by ammonium chloride lysis to reduce interference with subsequent assays or with flow cytometric analysis. The lysis can be performed as part of either wash step.