Isolating Specific Cell Subsets Directly from Whole Blood Without Columns or Magnets

The following procedure provides guidelines for isolating cells from whole blood 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.

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.

For a high-throughput protocol for NK cell and lymphocyte isolation, please see below.

1. Ensure that the blood 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 the sample and mix well.
3. Incubate 20 minutes at room temperature.
4. Dilute sample with an equal 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.
7. Remove the enriched cells from the density gradient medium: plasma interface.
8. 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.
9. Wash enriched cells with PBS + 2% FBS. Repeat wash.
10. 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.

Ficoll™ is a trademark of GE Healthcare Ltd. Lymphoprep™ is a trademark of Axis-Shield.

High-Throughput Protocol for NK Cell and Lymphocyte Isolation

Traditional methods for isolating lymphocytes from blood are laborious and time-consuming, requiring precision and technical expertise. These methods typically involve isolating peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation before enriching specific cell subpopulations using immunomagnetic column-based systems. Using these conventional methods, isolating highly purified natural killer (NK) cells from a 450 mL unit of blood is a lengthy process (taking up to 4 hours), and it is difficult to process multiple samples quickly and efficiently.1

The new RosetteSep™ and SepMate™ system allows faster and more efficient blood processing without compromising cell function or performance in downstream assays. At the University of Maryland School of Medicine, Dr. Ajay Jain and colleagues routinely isolate NK cells from large numbers of human samples. In order to streamline their workflow and achieve higher-throughput sample processing, Jain ' s lab has adopted the new RosetteSep™ and Sepmate™ cell isolation system in place of their previous, column-based method.

To learn more, read the case study from STEMCELL Technologies or see this Journal of Immunological Methods paper[1] for the detailed protocol.

So E.C. et al., J. Immunol. Methods. 2013. http://dx.doi.org/10.1016/j.jim.2013.05.001.