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

STEMCELL Technologies,Maria Thunoe

Abstract

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. Lymphoprep™), the immunorosettes pellet, leaving highly purified cells at the interface between the plasma and the density gradient medium.

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https://www.protocols.io/view/isolating-specific-cell-subsets-directly-from-who-nmadc2e
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Guidelines

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 allow 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.

See this Journal of Immunological Methods paper[1] for the detailed protocol.

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

Have a question about this protocol? Ask us by sending an email to techsupport@stemcell.com.

Before start

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

https://www.stemcell.com/products/brands/rosettesep.html

Protocol

Step 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 (15 - 25°C).

NOTES

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

Step 2.

Add RosetteSep™ cocktail to the sample and mix well.

Step 3.

Incubate 20 minutes at room temperature.

DURATION
00:20:00 : incubation

Step 4.

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

Step 5.

Layer the diluted sample on top of the density gradient medium using the recommended volumes (see note). Be careful to minimize mixing of the density gradient medium and the sample.

NOTES

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.

Step 6.

Centrifuge at 1200 x *g* for 20 minutes at room temperature, with the brake off.

DURATION
00:20:00 : Centrifugation

Step 7.

Remove the enriched cells from the density gradient medium:plasma interface.

Step 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.

Step 9.

Wash enriched cells with PBS + 2% FBS, centrifuge at 300 x *g* for 10 min, with brake on low. Discard supernatant. (1/2).

DURATION
00:10:00 : Centrifugation

NOTES

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.

Step 10.

Wash enriched cells with PBS + 2% FBS, centrifuge at 300 x *g* for 10 min, with brake on low. Discard supernatant. (2/2)

DURATION

00:10:00 : Centrifugation

NOTES

To remove residual RBCs, perform lysis with ammonium chloride as part of either wash step.

Step 11.

Use enriched cells as desired.

Warnings

Please refer to the SDS (Safety Data Sheet) for hazard information.