TECHNICAL BULLETIN

A Novel System for High-Throughput Cell Isolation Directly from Blood in 25 Minutes

This technical bulletin presents the new RosetteSep™ and SepMate™ system for fast, efficient and high-throughput cell isolation from whole blood samples. It features data from Dr. Ajay Jain's lab at the University of Maryland School of Medicine, which used this system to reduce NK cell isolation time from four hours to a single hour for a 450 mL unit of blood.

The Need For High-Throughput Cell Isolation

Immune cell isolation plays an important role in areas such as drug discovery and development, vaccine research, and translational immunology. For example, isolating immune cells is routinely necessary in order to evaluate the efficacy of immunostimulatory compounds, assess the immunogenicity of vaccine candidates and investigate interactions between the immune system and infectious agents.[1-4]

The movement towards more physiologically relevant assays based on primary human cells has created the need for a fast and efficient method of isolating immune cells from large numbers of whole blood samples.[2,4] To facilitate this type of high-throughput cell processing, STEMCELL Technologies Inc. has developed a new system for rapid and efficient cell isolation directly from whole blood in as little as 25 minutes.

The RosetteSep™ and SepMate™ System for Cell Isolation Directly from Whole Blood

The RosetteSep™ and SepMate™ system combines a unique immunodensity cell isolation reagent (RosetteSep™) with a specialized cell processing tube (SepMate™) to reduce the number of steps needed for cell isolation. This new system saves time, minimizes variability between users and allows efficient, high-throughput sample processing. It also minimizes the risk of activating or damaging cells by isolating untouched and highly purified cells without columns or immunomagnetic beads.

Cells isolated with the RosetteSep™ and SepMate™ system are immediately available for use in a variety of downstream assays such as cytotoxicity testing, compound screening, and other applications where it is important to obtain viable, functional cells with minimal manipulation. For more information, see page 3 of this technical bulletin.

Case Study: A High-Throughput Method for Isolation of Natural Killer Cells and Lymphocytes for Assessment of In Vitro Cytotoxicity

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 (DGC) 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 (Jain et al., in prep).

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.

Evaluation of the RosetteSep™ and SepMate™ System

Dr. Jain's group found that isolation of NK cells was significantly faster with the RosetteSep™ and SepMate™ system: a 450 mL unit of blood could be split into multiple samples and processed in a single hour, compared to four hours using DGC/immunomagnetic selection (Jain et al., in prep).

TOLL-FREE T. 1 800 667 0322 • T. +1 604 877 0713 • TECHSUPPORT@STEMCELL.COM • INFO@STEMCELL.COM
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Furthermore, NK cells isolated by RosetteSep™ and SepMate™ were highly purified (86.7% as assessed by flow cytometry) with intact cytolytic function. Using in vitro assays, Jain et al. showed that isolated cells retain the capacity to kill HT9 colon cancer cells or K563 leukemia cells (Figure 1).

Flow cytometric evaluation of NK cell activation markers demonstrated that NK cells isolated using RosetteSep™ and SepMate™ have similar expression profiles similar to cells isolated using DGC/immunomagnetic selection (Figure 2).

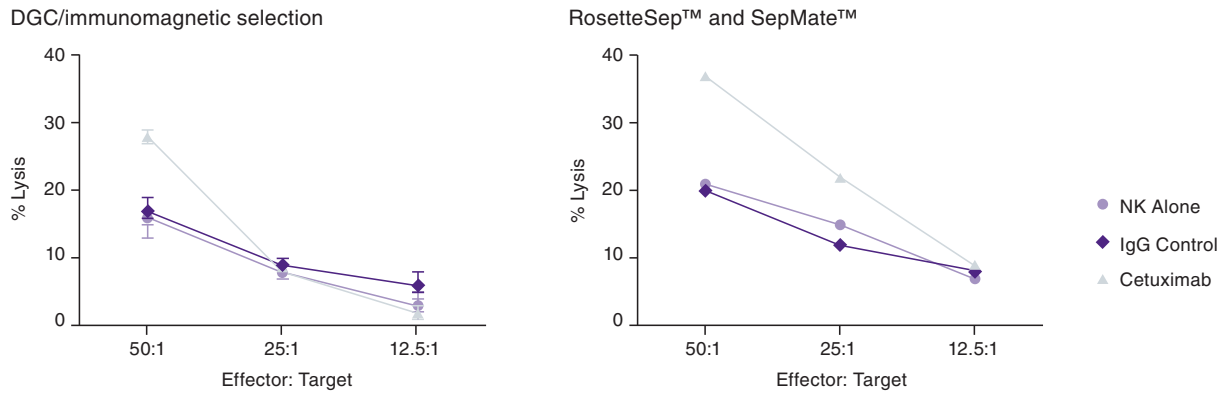

FIGURE 1. In vitro cytotoxicity against HT29 colon cancer cells and K562 target cells with NK cells isolated by DGC/immunomagnetic selection (left panel) versus RosetteSep™ and SepMate™ (right panel).

In vitro conditions include culture with media alone (open circles), 10 µg/mL human IgG1 isotype control (open triangles), or 10 µg/mL cetuximab (open diamonds). Figure courtesy of Dr. Ajay Jain.

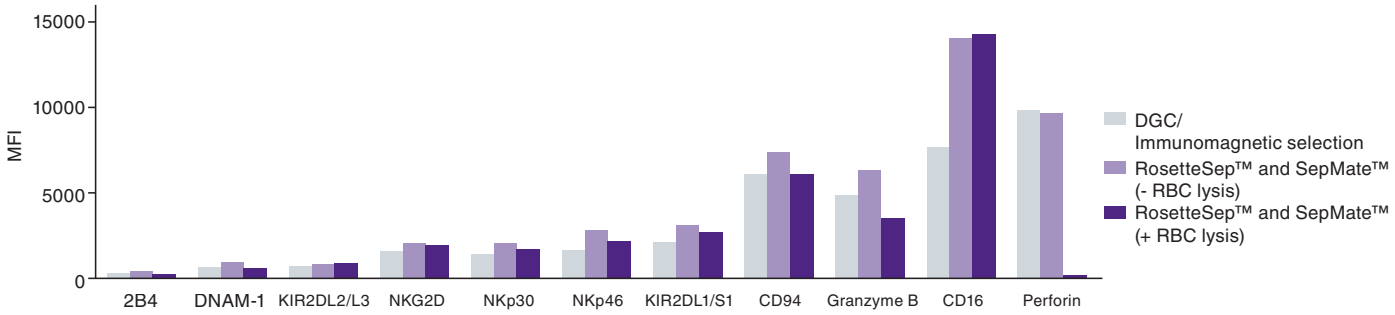

FIGURE 2. Surface expression of NK cell activation markers as evaluated by flow cytometry.

Expression is shown as mean fluorescence intensity (MFI). Figure courtesy of Dr. Ajay Jain.

"The SepMate™ tubes… provide a mechanical barrier between the purified effector cells and the density gradient medium. The desired cell populations remain above the barrier and can be poured out of the tube with no technical expertise. This method allows for the concurrent rapid purification of NK cells and $CD8^+$ and $CD4^+$ T cells from multiple human donors, which makes downstream applications (i.e. flow cytometry, ELISA, assessment of in vitro cytotoxicity) more practical to perform in a high-throughput manner."

– Jain et al., 2013.

Conclusions

The RosetteSep™ and SepMate™ system allows efficient negative selection of lymphocytes from whole blood in a quarter of the time needed for an alternative method, requires no technical expertise, and yields fully functional cells. This new system makes it possible to rapidly isolate NK cells or other immune cells from many samples at once, facilitating high-throughput cytotoxicity testing and other downstream assays.

For more information on the RosetteSep™ and SepMate™ procedure, see page 3.

How the RosetteSep™ and SepMate™ System Works

RosetteSep™ isolates highly purified cells directly from blood by crosslinking unwanted cells to red blood cells, forming immunorosettes (Figure 3). These immunorosettes pellet during density gradient centrifugation, leaving untouched and highly purified target cells at the interface between the plasma and the density gradient medium.

Using the new SepMate™ tube makes the RosetteSep™ procedure even faster and easier. The SepMate™ insert creates a physical barrier between the sample and density gradient medium, allowing the sample to be rapidly pipetted or poured into the tube. Centrifugation time is reduced to just 10 minutes, and purified target cells can simply be poured into a new tube. SepMate™ can also be used on its own for hassle-free PBMC isolation from whole blood in just 15 minutes.

Free samples and further information: **www.rosettesep.com**

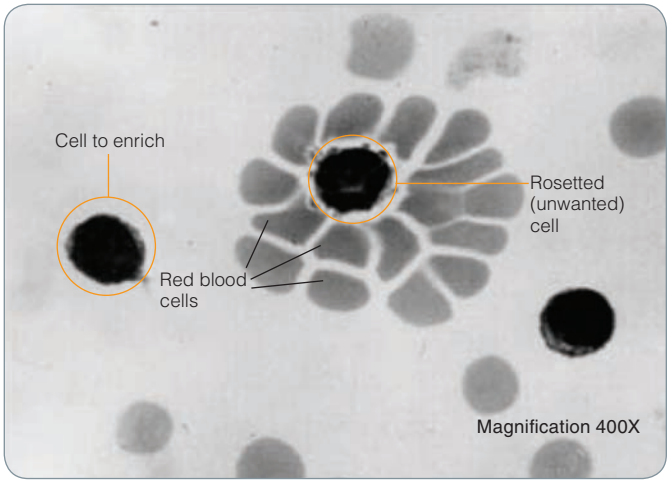

FIGURE 3. Image of a blood sample after addition of the RosetteSep™ cocktail, and prior to density gradient centrifugation.

Advantages of the RosetteSep™ and SepMate™ System

- **FAST AND EASY.** Isolate cells from whole blood in as little as 25 minutes with RosetteSep™ and SepMate™, or use RosetteSep™ on its own with standard density gradient centrifugation.
- **NO SPECIAL TRAINING OR EQUIPMENT.** RosetteSep™ and SepMate™ can be used by anyone with minimal training. No columns, magnets or other special equipment are required.
- **HIGHLY VIABLE AND FUNCTIONAL CELLS.** Isolated cells are functional, flow cytometry-compatible, and unlabeled with antibodies or magnetic beads.

The Novel RosetteSep™ and SepMate™ Procedure

Incubate the blood sample with RosetteSep™ for 10 minutes at room temperature.

Add density gradient medium to SepMate™ tube, then rapidly pipet or pour the blood sample directly over the insert.

After a 10-minute centrifugation with the brake on, simply pour highly purified target cells into a new tube.

25 Minutes

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RosetteSep™ Product Listing

Human Negative Selection

CELL TYPE TO ENRICH	CATALOG #		PERFORMANCE DATA
	2 mL[1]	10 mL[2]	% Purity
T Cells	15021	15061	90 – 97
CD4+ T Cells	15022	15062	89 – 99
CD8+ T Cells	15023	15063	75 – 93
B Cells	15024	15064	75 – 91
NK Cells	15025	15065	64 – 84
Total Lymphocytes	15223	15263	92 – 96[3]
Lymphoid	-	15271HLA[4]	96
Myeloid	-	15272HLA[4]	97
Monocytes	15028	15068	62 – 80
Cell Depletion Cocktails[5]			Depletion
CD3+ Cell Depletion	15621	15661	3.0 log
CD4+ Cell Depletion	15622	15662	2.0 log
CD8+ Cell Depletion	15623	15663	2.0 log
CD45 Depletion (Epithelial Tumor Cell Enrichment)	15122	15162	3.6 log
Granulocyte Depletion (CD66b)	15624	15664	-
Monocyte Depletion (CD36)	15628	15668	2.8 log
IgE Depletion	15230	-	-

1. For labeling 40 mL of blood.
2. For labeling 200 mL of blood.
3. For CD2+ or CD19+ cells.
4. This product carries the CE marking.
5. Can be added to a standard RosetteSep™ cocktail, if not already present, or used alone.

SepMate™ Product Listing

PRODUCT NAME	CATALOG #	QUANTITY
SepMate™-50	15450	20 tubes
	15460	100 tubes

References

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Further Reading: Selected Recent RosetteSep™ Cytotoxicity Publications

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22. Trifari S, et al. Nature Immunology 10: 864-871, 2009
23. Haniffa M, et al. J Exp Med 206(2): 371-385, 2009
24. Critchley RJ, et al. PNAS 106(22): 9010-9015, 2009
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