

What is RosetteSep®?

RosetteSep® is a rapid cell separation procedure for the isolation of highly purified cells directly from human whole blood. RosetteSep® turns a simple density centrifugation step into a specific antibody-mediated cell enrichment procedure with StemCell's tetrameric antibody complex (TAC) technology (Figure 1). The RosetteSep® cocktail crosslinks unwanted cells to multiple red blood cells already present in the sample, forming immunorosettes (Figure 2). When centrifuged over the appropriate density medium (e.g. Ficoll™), the unwanted (rosetted) cells pellet along with the red blood cells, leaving the desired cells untouched and highly enriched at the density medium:plasma interface. Desired cells are never labeled with antibody and are immediately ready for any application.

Advantages

- No magnets, no columns, no special equipment!
- Just a 20 minute incubation prior to a standard Ficoll™ spin
- Easy - one-step cell enrichment directly from whole blood
- Isolation performed at room temperature
- High recovery - no post-Ficoll™ cell losses
- Negative selection - cells are unlabeled and ready for immediate use
- Untouched cells yield high-quality RNA for molecular studies.¹⁻³
- Safe - sample handling is minimized
- 2 year shelf-life, minimizing reagent validations

Cell Enrichment Directly from Whole Blood

- T cells
- CD4+ T cells
- CD8+ T cells
- B cells
- NK cells
- Total lymphocytes
- Multiple myeloma cells
- Monocytes
- Progenitors
- Circulating epithelial tumor cells
- Granulocytes
- Mesenchymal stem cells
- and more...

Plus! Depletion of:

- Granulocytes
- Monocytes
- T cells & subsets

Figure 1. Illustration Showing Rosette of Unwanted Cell and RBCs Formed by RosetteSep® Tetrameric Antibody Complexes.

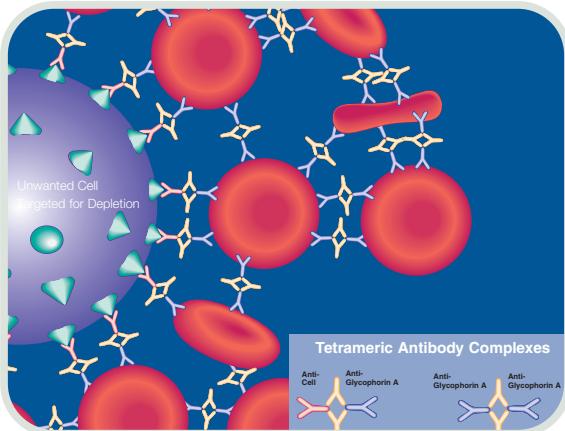
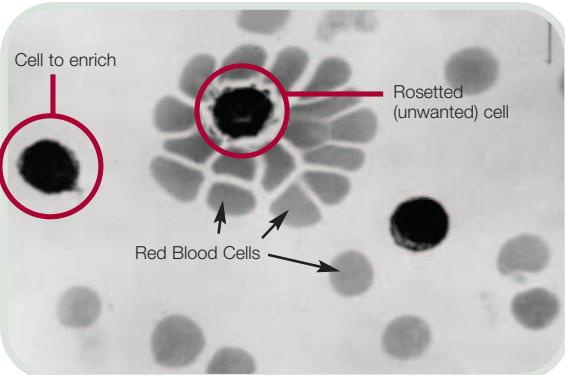
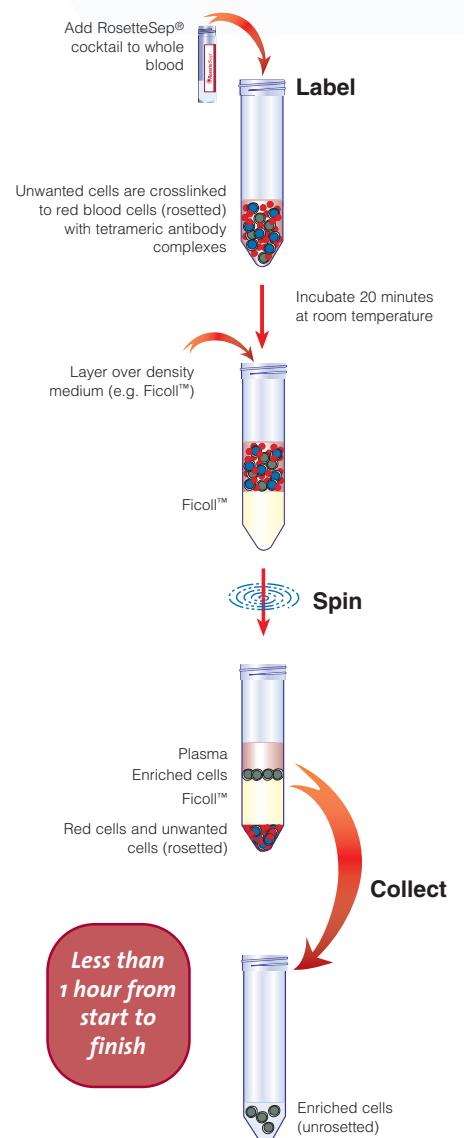


Figure 2. Picture of a blood sample after addition of the RosetteSep® cocktail, and prior to centrifugation over Ficoll™. Magnification 400X.



RosetteSep® Procedure



Examples of RosetteSep® Applications

Cocktails can be used directly with BD Vacutainer® CPT™-Cell Preparation Tubes

BD Vacutainer® CPT™ - Cell Preparation Tubes* are evacuated blood collection tubes, containing a density gradient liquid below a gel insert, and an anticoagulant above. The RosetteSep® procedure can be performed directly in the CPT, minimizing sample manipulation. The gel insert in the CPT keeps the sample and the density gradient layer apart before and after centrifugation, avoiding the layering step and facilitating removal of the enriched cells.

To perform a RosetteSep® separation in a CPT, the RosetteSep® cocktail is added to the blood sample directly in the CPT. After incubation and centrifugation, the enriched cells are recovered by pipetting or pouring from the CPT. This approach may be advantageous when minimal sample handling is preferred, such as when processing infectious samples.

*Available from BD (www.vmdp.com).
BD Vacutainer® and CPT™ are trademarks of Becton, Dickinson and Company.

Isolate cells for HLA analysis

After RosetteSep® enrichment, cells are immediately usable for serological HLA typing, CDC crossmatching, flow crossmatching, and for all flow cytometry or DNA applications. Because RosetteSep® does not interfere with subsequent analysis and is gentle on cells, this procedure can reduce the risk of false positive data in HLA typing.

Selected ASHI Abstracts

Dunn DM, Thurmon MN, Sypert CL: Cell preparation that can be used for serological HLA typing, crossmatching, and flow crossmatching. ASHI Abstract #79, 2003

Gautreaux MD, Haywood MJ, James CM, White CD: Evaluation of RosetteSep™ with modification of test protocol to increase yield and reduce red blood cell contamination. ASHI Abstract #120, 2003

Martin C, McKie J, Hazy L, Shumway W, LeFor W: Purified T and B cell preparations obtained using RosetteSep cell isolation reagents: suitability for use in cytotoxic assays. ASHI Abstract #96, 2002

Isolate specific T cell subsets: Example - Regulatory CD4+CD25+T Cells

Use EasySep® to select specific subsets of cells previously enriched with RosetteSep®.
To isolate CD4+CD25+ T cells, first enrich CD4+ T cells with RosetteSep®, then select CD4+CD25+ T cells using the EasySep® CD25 Selection Cocktail. Activated non-CD4+ T cells are removed during the RosetteSep® procedure prior to CD25 positive selection. For more information on EasySep®, please visit www.stemcell.com.

Ficoll™ and Ficoll-Paque™ PLUS are trademarks of GE HealthCare Ltd.

Did you know..?

RosetteSep® can be used with samples other than whole blood. With the appropriate procedure, cells can be enriched from buffy coat, bone marrow, cord blood, leukaphereses, spleen, lymph node and frozen blood. Contact us for details.

Did you know..?

RosetteSep® Granulocyte Depletion reagent can be used to eliminate granulocytes from older blood samples

Ask us about a RosetteSep®
demonstration
in your lab!

RosetteSep® Products

Cell Enrichment Cocktails	2 mL*	10 mL**
T Cells	15021	15061
CD4+ T Cells	15022	15062
CD8+ T Cells	15023	15063
B Cells	15024	15064
NK Cells	15025	15065
Total Lymphocytes	15223	15263
Monocytes	15028	15068
Granulocytes [†]	15121	15161
Progenitor Cells from Cord Blood	15026	15066
De-Bulking Cord Blood for Freezing	15126	15166
Progenitor Cells from Bone Marrow	15027	15067
Mesenchymal Stem Cells from Bone Marrow	15128	15168
Multiple Myeloma (B and Plasma) Cells	15129	15169
Circulating Epithelial Tumor Cells (CD45 Depletion)	15122	15162
Circulating Epithelial Tumor Cells (Extensive Enrichment)	15127	15167
Cell Depletion Cocktails***		
Catalog #		
	2 mL*	10 mL**
CD3+ Cell Depletion	15621	15661
CD4+ Cell Depletion	15622	15662
CD8+ Cell Depletion	15623	15663
Granulocyte Depletion (CD66b)	15624	15664
Monocyte Depletion (CD36)	15628	15668
IgE Depletion	15230	-
Support Reagents		
Catalog #		
	100 mL	500 mL
Ficoll-Paque™ Plus	07907	07957
RosetteSep® DM-L	15705	-
HetaSep®	07906	-

* for labeling 40 mL of blood

** for labeling 200 mL of blood

*** Can be added to a standard RosetteSep® cocktail, if not already present.

† Kit contains required density medium.

Selected RosetteSep® Publications (with reported purities where available)

1. Cobb *et al.*, Proc Natl Acad Sci USA 102: 4801-4806, 2005. T cell and monocyte enrichment for microarray analysis. Purity 80-95%.
2. Rouhainen *et al.*, Blood 104: 1174-1182, 2004. Monocyte enrichment for cell migration and RT-PCR studies.
3. Sasaki *et al.*, Blood 105: 1204-1213, 2005. CD4+ T cell enrichment for microarray analysis. Purity >90%.
4. Dyugovskaya *et al.*, Am J Respir Crit Care Med 168:242-249, 2003. $\gamma\delta$ T cell enrichment for functional and molecular studies.
5. Poggi *et al.*, J Immunol 174: 2653-2660, 2005. NK cell enrichment for functional and molecular studies.
6. Frelin *et al.*, Blood 105: 804-811, 2005. Hematopoietic progenitor cell enrichment for cytotoxicity studies.
7. Castriconi *et al.*, Proc Natl Acad Sci USA 101: 12640-12645, 2004. Neuroblastoma cell enrichment from bone marrow for molecular characterization studies; NK cell enrichment for functional studies.
8. Parsons *et al.*, Blood 104: 2736-2738, 2004. Mesenchymal stem cell enrichment from fetal bone marrow for culture and virus susceptibility studies. Purity >99% after 4 days culture.
9. Vasil *et al.*, J Immunol 174: 2376-2386, 2005. T cell and B cell enrichment for cytokine activation studies. Purity 95-97% (T cells), >90% (B cells).

RosetteSep® Custom Cocktails

(Catalog #15309)

In addition to our standard RosetteSep® cocktails, custom cocktails may be easily prepared to meet your unique cell separation needs. Design your own custom cocktail, or contact us for assistance.

RosetteSep® custom cocktails have been used by researchers to enrich various cell subsets, such as $\gamma\delta$ T cells⁴, naïve, memory, or resting T cells, dendritic cells, etc.

Anti-Human Antibodies Available For Use In Custom Cocktails

CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD14, CD15, CD16, CD19, CD20, CD24, CD25, CD27, CD29, CD33, CD34, CD36, CD38, CD41, CD45, CD45RA, CD45RO, CD56, CD66b, CD66e, CD69, CD124, HLA-DR, IgE, breast carcinoma, TCR $\alpha\beta$, TCR $\gamma\delta$.

Other antibodies are available upon request.

RosetteSep® is an extremely flexible system, which can also be used with your own "in-house" IgG₁ mouse monoclonal antibodies.

Please contact us for assistance in optimizing your cell separation procedures.

POWERFUL CELL
SEPARATION WITH A
SIMPLE SPIN!

 **RosetteSep®**

Obtain highly purified cells from whole blood faster and more easily than ever before!

