

STEMSEP® ABBREVIATED PROCEDURE:

Refer to StemSep® Cell Separation Technical Manual for additional information. www.stemcell.com/technical/28416_ssmanual.pdf

1. Prepare mononuclear cell suspension at a concentration of 5×10^7 cells/mL or within the acceptable range of $2 - 8 \times 10^7$ cells/mL in separation medium (see Notes and Tips).
2. Add StemSep® Enrichment Cocktail at 100 μ L/mL cells (e.g. for 1 mL of cells add 100 μ L of cocktail). Mix well.
3. Incubate on ice for 30 minutes or at room temperature for 15 minutes.
4. Add Magnetic Colloid at 60 μ L/mL of cells (e.g. for 1 mL of cells add 60 μ L of colloid). Mix well.
5. Incubate on ice for 30 minutes or at room temperature for 15 minutes.
6. During incubation prepare column (refer to diagrams opposite) as follows:
 - a) Using Table 3 (see Notes and Tips) determine the appropriate column size based on cell number.
Note: Do not insert column from the front of the magnet. Lower column slowly from above down into the gap of magnet.
 - b) Gravity Feed - Place column in magnet and assemble. Prime with priming medium (see Notes and Tips) from the bottom up by depressing plunger of side syringe slowly.** Check for air bubbles. Proceed with 6d.
 - c) Pump Feed - Place column in magnet and assemble. Prime with priming medium (see Notes and Tips) from the bottom up at appropriate speed (see Table 1). Check for air bubbles. Proceed with 6d.
 - d) Wash from the top down with appropriate volume of separation medium (see Table 2).
7. Load sample.
8. Wash from the top down with separation medium, collecting the sample volume plus the appropriate column wash volume as flowthrough (see Table 2). The enriched cells are now ready for use.

TABLE 1. FLOW RATES AND PUMP SETTINGS

COLUMN SIZE	PRIMING		LOADING SAMPLE AND WASHING	
	mL/min	pump setting*	mL/min	pump setting*
1.0"	2.0	10.0	5.0	27.0
0.6"	0.6	3.0	2.0	10.0
0.5"	0.3	1.5	1.0	5.0
0.3"	0.2	1.0	0.6	3.0

*Pump setting for 4-channel pump supplied by STEMCELL Technologies only.

TABLE 2. COLUMN WASH VOLUME

COLUMN SIZE	COLUMN WASH VOLUME
1.0"	90 mL
0.6"	25 mL
0.5"	15 mL
0.3"	8 mL
0.1"	1.5 mL

PROCEDURE



Negative Selection

StemSep®

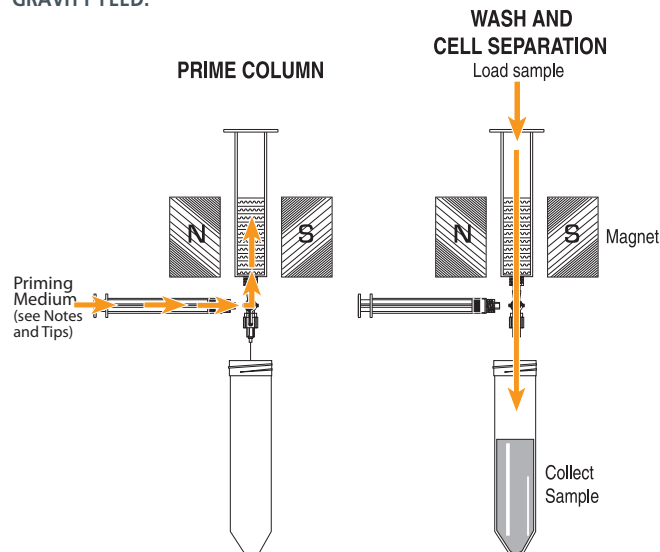


VERSION 2.2.1

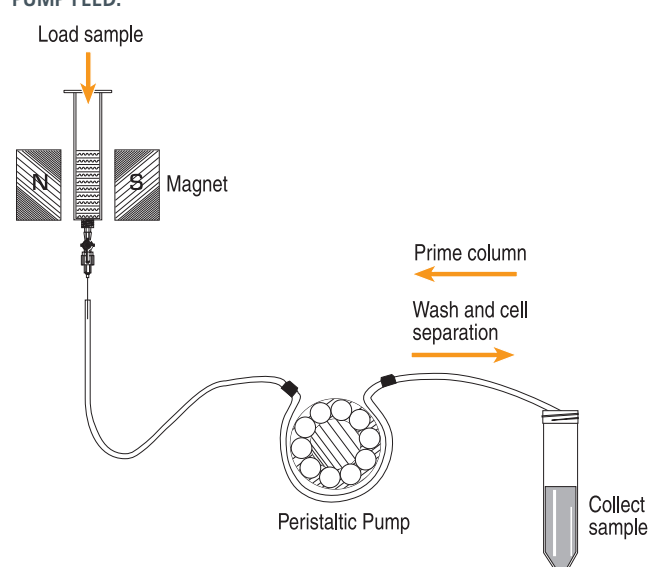
**HUMAN
NK CELL
ENRICHMENT
KIT**

CATALOG #14055 (14065)

GRAVITY FEED:



PUMP FEED:



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FOR RESEARCH USE ONLY

FEBRUARY 2009

#28884

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CATALOG #14055 (14065)

 For labeling 10^9 (5×10^9) total cells

Components:

- StemSep® Human NK Cell Enrichment Cocktail 2.0 mL (5×2.0 mL)
- Magnetic Colloid 1.5 mL (4×1.5 mL)


PRODUCT INFORMATION SHEET
REQUIRED EQUIPMENT:

StemSep® Magnet (Catalog #11030, 11050, 11060, or 11070) or a magnet with the strength of at least 0.5 Tesla, and StemSep® Negative Selection Columns (see Table 3, Notes and Tips).

PRODUCT DESCRIPTION AND APPLICATIONS:

The StemSep® Human NK Cell Enrichment Kit is designed to enrich NK cells from a peripheral blood mononuclear cell suspension.

STEMSEP® LABELING OF HUMAN CELLS:

Target cells are specifically labeled with colloidal magnetic dextran iron particles using bispecific tetrameric antibody complexes (TAC). These complexes recognize both dextran and the target cell surface antigen (Figure 1). Magnetically labeled cells are then separated from unlabeled cells by passing them through a magnetic separation column placed in a magnet.

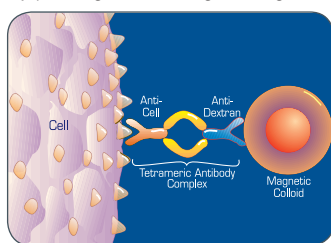


FIGURE 1.
Schematic Drawing of StemSep® TAC
Magnetic Labeling of Human Cells.

NOTES AND TIPS:
COLUMN PREPARATION:

- Use the appropriate column size (see Table 3).
- Check all the connections during priming and washing to ensure they do not leak.
- Prime the column from the bottom up.
- Use priming medium (see below) to prime the column.
- Ensure that there are no air bubbles in the column.
- Use separation medium (see below) to wash the column. The protein present in the wash solution prevents cells from binding non-specifically to the column.
- Ensure that the column does not run dry at any time.

PREPARING A MONONUCLEAR CELL SUSPENSION: Prepare a mononuclear cell suspension from peripheral blood by Ficoll-Paque™ PLUS density separation (Catalog #07957). For previously frozen mononuclear cells, we recommend the addition of 100 mg/mL DNase (Catalog #07900) prior to labeling and separation. Excessively clumpy suspensions may be passed through a 30 - 70 µm mesh nylon strainer.

RECOMMENDED MEDIA: Using degassed media reduces the chance of developing air bubbles in the column. Air bubbles cause channeling in the column reducing the capacity of the column and potentially compromising purity. Media should be Ca^{++} and Mg^{++} free.

- **PRIMING MEDIUM:** Use PBS (Catalog #37350), either at room temperature or degassed, without serum or other protein.
- **SEPARATION MEDIUM:** Use PBS + 2% FBS (Catalog #07905).

ASSESSING PURITY. Purity of NK cells can be measured using flow cytometry by staining with a fluorochrome-conjugated anti-CD56 antibody (e.g. anti-CD56 PE, Catalog #10526).

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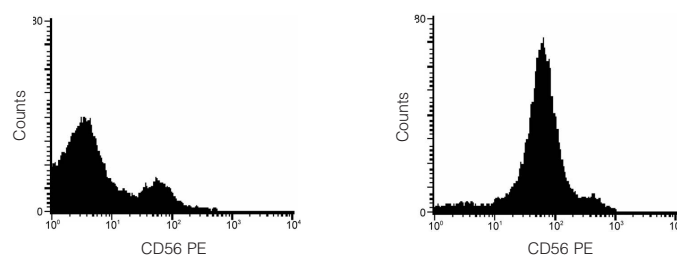
TABLE 3. RECOMMENDED NUMBER OF HUMAN MONONUCLEAR CELLS IN THE START SUSPENSION FOR VARIOUS COLUMN SIZES

COLUMN SIZE	CATALOG # GRAVITY PUMP	COLUMN CAPACITY BASED ON CELL NUMBER	WILL FIT MAGNET SIZE
1.0"	- 12470	2×10^9 - 2.0×10^{10}	black
0.6"	12061 12062	10^8 - 1.5×10^9	green, blue, black
0.5"	12051 12052	5×10^7 - 3×10^8	green, blue, black
0.3"	12031 12032	2×10^7 - 10^8	all sizes
0.1"	12021 -	10^5 - 2×10^7	red, green

TYPICAL STEMSEP® NK CELL ENRICHMENT PROFILE:

Start: 19% CD56⁺ Cells

Enriched: 91% CD56⁺ Cells



Starting with a previously frozen peripheral blood mononuclear cell suspension, the CD56⁺ cell content of the enriched fraction typically ranges from 80 - 94%.

COMPONENT DESCRIPTIONS:
STEMSEP® HUMAN NK CELL ENRICHMENT COCKTAIL CODE #14055C

This cocktail contains a combination of monoclonal antibodies purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in bispecific TAC which are directed against cell surface antigens on human hematopoietic cells (CD3, CD4, CD14, CD19, CD66b, glycophorin A) and dextran. The mouse monoclonal antibody subclass is IgG₁. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable. Supplied in phosphate buffered saline.

MAGNETIC COLLOID

CODE #10051

A colloidal suspension of magnetic dextran iron particles in USP saline, pH 7.0 - 7.5.

STABILITY AND STORAGE:
STEMSEP® HUMAN NK CELL ENRICHMENT COCKTAIL

Stable at 2 - 8°C for 2 years. Do not freeze. This product has been sterility tested.

MAGNETIC COLLOID

This product is shipped at room temperature. Once opened, stable at 2 - 8°C for 6 weeks. Stable at -20°C for 1 year. Repeated freezing and thawing is possible but not recommended. Vortex before re-freezing. This product has been sterility tested.

See Material Safety Data Sheet for more information.



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