STEMSEP® ABBREVIATED PROCEDURE:

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

1. Prepare cells at a concentration of 5 x 10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL in separation medium (see Notes and Tips).
2. Add normal rat serum to cell suspension at 50 µL/mL of cells (e.g. for 1 mL of cells, add 50 µL of serum). Incubate at 4°C (refrigerator) for 15 minutes.
3. Add StemSep® Enrichment Cocktail at 35 µL/mL of cells (e.g. for 1 mL of cells add 35 µL of cocktail). Mix well.
4. Incubate at 4°C (refrigerator) for 15 minutes.
5. Wash and resuspend cells (2 - 8 x 10^7 cells/mL) in separation medium.
6. Add Anti-Biotin TAC at a concentration of 100 µL/mL of cells (e.g. for 1 mL of cells add 100 µL of anti-biotin TAC). Mix well.
7. Incubate at 4°C (refrigerator) for 15 minutes.
8. Add Magnetic Colloid at a concentration of 60 µL/mL of cells (e.g. for 1 mL of cells add 60 µL of colloid). Mix well.
9. Incubate at 4°C (refrigerator) for 15 minutes.
10. 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 10d.
    ****Note:** 0.1" column is primed quickly.
    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 10d.
    d) Wash from the top down with appropriate volume of separation medium (see Table 2).
    Note: Do not let column run dry at anytime during priming, loading or washing of the column.
11. Load sample.
12. 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*
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
0.6"	25 mL
0.5"	15 mL
0.3"	8 mL
0.1"	1.5 mL

PROCEDURE

Negative Selection

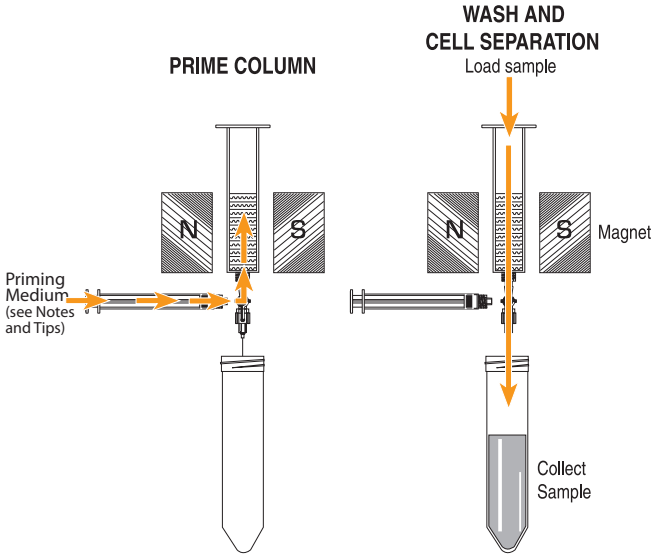
StemSep®

MOUSE HEMATOPOIETIC PROGENITOR CELL ENRICHMENT KIT

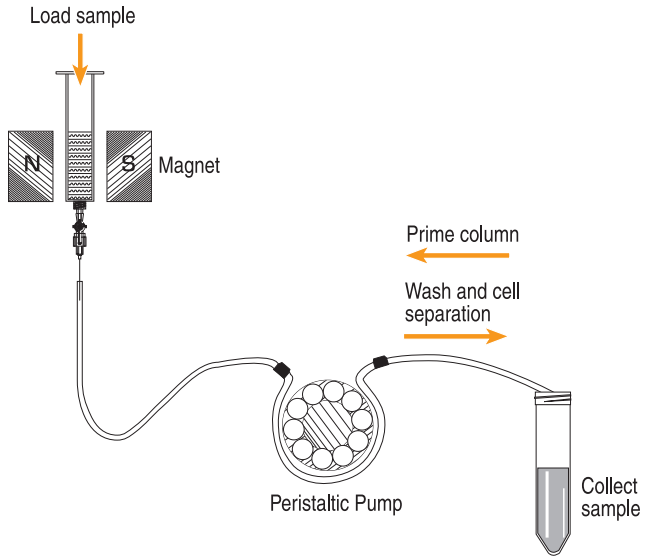
VERSION 3.1.1

CATALOG #13056 (13066)

GRAVITY FEED:



PUMP FEED:



STEMCELL TECHNOLOGIES

In North America
Tel: 1.604.877.0713
Fax: 1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax 1.800.567.2899
e-mail: info@stemcell.com
WWW.STEMCELL.COM

In Europe
Tel: +33.(0).4.76.04.75.30
Fax: +33.(0).4.76.18.99.63
e-mail: info.eu@stemcell.com

In Australia
Toll Free Tel: 1.800.060.350
Fax: +61.(03).9338.4320
e-mail: info.aus@stemcell.com

FEBRUARY 2009

FOR RESEARCH USE ONLY

#28906

Printed on recycled paper.

CATALOG #13056 (13066) For labeling 10^9 (5×10^9) total cells

Components:

• StemSep® Mouse Hematopoietic Progenitor Cell Enrichment Cocktail	700 µL (5 x 700 µL)
• Anti-Biotin TAC	2.0 mL (5 x 2.0 mL)
• Magnetic Colloid	1.5 mL (5 x 1.5 mL)
• Rat Serum	2.0 mL (5 x 2.0 mL)

StemSep® Negative Selection

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® Mouse Hematopoietic Progenitor Cell Enrichment Kit is designed to highly enrich hematopoietic progenitor cells (HSCs) from suspensions of mouse bone marrow. The recovered hematopoietic progenitor cells (HSCs) have not been labeled with antibody.

STEMSEP® LABELING OF MOUSE CELLS:

Antigens on the surface of unwanted cells are first labeled with biotinylated monoclonal antibodies (Figure 1). Cells are then linked to magnetic dextran iron particles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both biotin and dextran on the magnetic colloid/nanoparticle (Figure 1). Magnetically labeled cells are then separated from unlabeled cells by passing them through a magnetic separation column placed in a magnet.

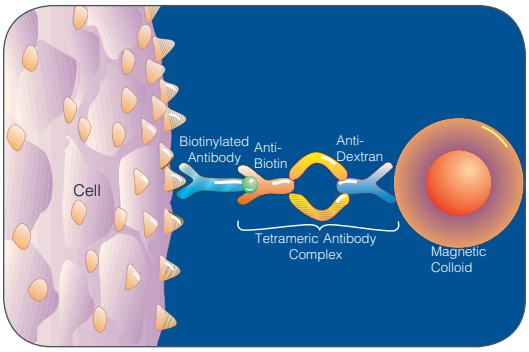


FIGURE 1. Schematic Drawing of StemSep® TAC Magnetic Labeling of Mouse Cells.

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 separation medium prevents cells from binding non-specifically to the column.
- Ensure that the column does not run dry at any time.

PREPARING A SINGLE CELL SUSPENSION. Bone Marrow: Flush bone marrow cells from femur and tibia into separation medium using a syringe equipped with a 23-gauge needle. Disperse clumps by gently passing the cell suspension through the syringe several times. Remove remaining clumps of cells and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge, discard supernatant and resuspend cells at 5×10^7/mL (a range of $2 - 8 \times 10^7$/mL is acceptable) in separation medium.

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) or Hank's (Catalog #37250) + 2% FBS.

ASSESSING PURITY. HSCs can be measured using flow cytometry by staining simultaneously with a combination of FITC labeled lineage-specific antibodies (e.g. anti-CD3, anti-CD45R/B220, anti-Gr-1, anti-CD11b, anti-TER119), anti-SCA-1PE (Catalog #10816) and anti-c-Kit APC (Catalog #10850). The more primitive progenitors can be defined as Lin-SCA1+c-Kit+*. SCA-1 expression on HSCs is variable between mouse strains.

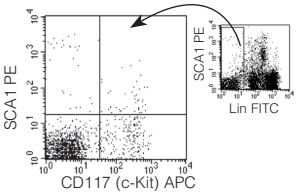
* Refer to Technical Bulletin at www.stemcell.com/technical/bulletins.asp.

TABLE 3. COLUMN CAPACITY: RECOMMENDED NUMBER OF MOUSE NUCLEATED CELLS IN THE START SUSPENSION FOR VARIOUS COLUMN SIZES

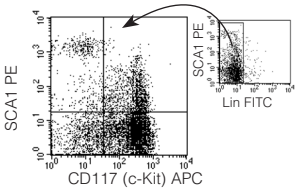
COLUMN SIZE	CATALOG # GRAVITY PUMP		COLUMN CAPACITY BASED ON CELL NUMBER	WILL FIT MAGNET SIZE
0.6"	12061	12062	10^8 - 1.5×10^9	green, blue, black
0.5"	12051	12052	5×10^7 - 3×10^8	green, blue, black
0.3"	12031	12032	2×10^7 - 10^8	all sizes
0.1"	12021	-	10^5 - 2×10^7	red, green

TYPICAL STEMSEP® MOUSE HEMATOPOIETIC PROGENITOR CELL ENRICHMENT PROFILE:

Start: 0.4% Lin$^-$SCA1$^+$c-Kit$^+$ Cells
2.0% Lin$^-$SCA1$^-$c-Kit$^+$ Cells



Enriched: 15.8% Lin$^-$SCA1$^+$c-Kit$^+$ Cells
64.3% Lin$^-$SCA1$^-$c-Kit$^+$ Cells



COMPONENT DESCRIPTIONS:

STEMSEP® MOUSE HEMATOPOIETIC PROGENITOR CELL ENRICHMENT COCKTAIL — CODE #13056C

This cocktail contains a combination of biotinylated monoclonal antibodies purified from rat ascites fluid or hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are directed against cell surface antigens on mouse hematopoietic cells (CD5 (Ly-1), CD11b (Mac-1), CD45R/B220, Ly-6G/C (Gr-1), Neutrophils (7-4), TER119). 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 with 0.1% BSA.

ANTI-BIOTIN TAC — CODE #13050C

A combination of mouse and rat monoclonal antibodies purified using Protein G Sepharose. The monoclonal antibody subclass is IgG_1. The antibodies form tetrameric antibody complexes (TAC) which are directed against both biotin and dextran. 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.

RAT SERUM — CODE #13551

Serum to prevent non-specific binding of rat antibodies to mouse cells. Certified mycoplasma-free.

STABILITY AND STORAGE:

STEMSEP® MOUSE HEMATOPOIETIC PROGENITOR CELL ENRICHMENT COCKTAIL AND ANTI-BIOTIN TAC

Stable at 2 - 8°C for 1 year and 2 years, respectively. Do not freeze. These products have 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.

RAT SERUM

Stable at least 2 years when stored at -20°C. Stable for 2 months when stored at 2 - 8°C. This product has been sterility tested.

See Material Safety Data Sheet for more information.

STEMCELL TECHNOLOGIES

In North America
Tel: 1.604.877.0713
Fax: 1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax 1.800.567.2899
e-mail: info@stemcell.com
WWW.STEMCELL.COM

In Europe
Tel: +33.(0).4.76.04.75.30
Fax: +33.(0).4.76.18.99.63
e-mail: info.eu@stemcell.com

In Australia
Toll Free Tel: 1.800.060.350
Fax: +61.(03).9338.4320
e-mail: info.aus@stemcell.com

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