Helpful Hints

To ensure optimal results when using StemSep™, follow these suggestions:

Reagents

- Store the reagents correctly.
  Do not freeze tetrameric antibody complex; store at 4°C.
  The magnetic colloid may be stored for up to six weeks at 4°C, or frozen at -20°C for up to one year. Repeated freezing and thawing is possible but not recommended. If freezing, vortex vigorously just prior to freezing. If particulate matter is visible when thawing, vortex and store at 4°C for 24 hours. Small particulate matter can be removed by filtering through a 0.2 µm filter.
- Use buffered salt solutions without Ca^{++} or Mg^{++} with 2 to 6% FBS.
- Add EDTA to a final concentration of 1 mM to recommended medium when enriching for adherent cells such as dendritic cells.

Column Preparation

- Check all the connections during priming and washing to ensure they do not leak.
- Prime the column from the bottom up.
- Use **PBS without FBS** or other protein (serum) to prime the column.
- Ensure that there are no air bubbles in the column.
- Use PBS with FBS, or Hank's with FBS to wash the column. For enrichment of dendritic cells, add EDTA to a final concentration of 1 mM.
  The protein in the wash solution blocks any protein binding sites on the mesh in the column, thus preventing cells from binding non-specifically to the column.
- Ensure that the column does not run dry at any time.

Cell Labeling

- Add normal rat serum to cells prior to labeling.
  The normal rat serum blocks Fc receptors on mouse cells, thereby preventing the non-specific binding of rat antibodies. This requires at least 15 minutes incubation prior to adding the antibody cocktail.
- Incubate cells at 4 to 8°C, in the refrigerator, not on ice.

StemSep®
Procedure - Mouse Cells

Hematopoietic Progenitor Enrichment and Enrichment of Human Cells from Mouse/Human Chimeras

Typically isolated from bone marrow.

Lymphocyte (T, B, NK) and Dendritic Cell Enrichment

Typically isolated from spleen cell suspensions.

Expected Cell Numbers

Bone Marrow: $1 - 2 \times 10^7$ per femur
6×10^6 per tibia
$3 - 5 \times 10^7$ per mouse

Spleen: 1×10^8 per mouse

Recommended Medium: Buffered salt solutions without Ca^{++} or Mg^{++}, such as PBS, modified with 2% fetal bovine serum (FBS). The addition of EDTA is suggested to improve recovery of adherent cells (dendritic cells: 1 mM).

Table 1. Optimum Number of Mouse Nucleated Cells in the Start Suspension for Various Column Sizes

Column Size	Optimum # of Cells	Extended Range of Cell # for Cell Enrichment
1.0"	10^{10}	2×10^9 - 1.5×10^{10}
0.6"	5×10^8	10^8 - 1.5×10^9
0.5"	10^8	5×10^7 - 3×10^8
0.3"	5×10^7	2×10^7 - 8×10^7
0.1"	10^6 - 10^7	10^5 - 2×10^7

Abbreviated Procedure - Mouse Cells

Refer to Manual for Further Detail.

1. Resuspend cells at 5x10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL in recommended medium (see previous page).
2. Add normal rat serum to cell suspension (final concentration 5%); incubate at 4°C (refrigerator) for 15 minutes.
3. Add the appropriate volume of antibody cocktail. Please use the volume indicated on the vial label or PIS corresponding to your 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 recommended medium.
6. Add 100 µL of anti-biotin tetrameric antibody complexes/mL cells. Mix well.
7. Incubate at 4°C (refrigerator) for 15 minutes.
8. Add 60 µL of magnetic colloid/mL cells. Mix well.
9. Incubate at 4°C (refrigerator) for 15 minutes.
10. Prepare column as follows (refer to diagrams on opposite page):
    a) Pump Feed - Assemble column and prime with PBS (no protein) from the bottom up at appropriate speed (see Table 2). Check for air bubbles. Place in magnet. Proceed with 10c.

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

Table 2. Flow Rates and Pump Settings

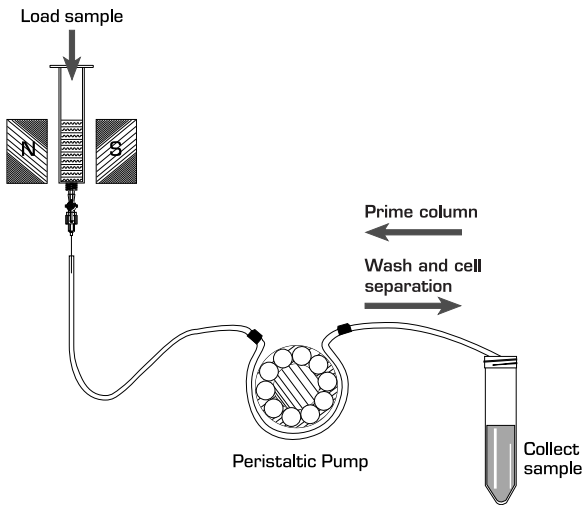
*Pump setting for 4-channel pump supplied by StemCell Technologies only.

    b) Gravity Feed - Place column in magnet and assemble. Prime with PBS (no protein) from the bottom up by depressing plunger of side syringe slowly.** Check for air bubbles. Proceed with 10c.
    **Note: 0.1" column is primed quickly.
    c) Wash (top down) with 3X column volume of recommended medium (see Table 3).
11. Load sample. Wash through with recommended medium, collecting sample volume plus 3X column volume as flowthrough (see Table 3).

Column Size	3X Column Volume
0.6"	25 mL
0.5"	15 mL
0.3"	8 mL
0.1"	1.5 mL

Prepare Column

Pump Feed:



Gravity Feed: