

## 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<sup>++</sup> or Mg<sup>++</sup> with 2 to 6% FBS.

### 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.  
*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.



### Lymphocyte (T, CD4<sup>+</sup> T, CD8<sup>+</sup> T) Enrichment

Typically isolated from spleen cell suspensions.

### Expected Cell Numbers

Spleen: 5 x 10<sup>8</sup> per rat

**Recommended Medium:** Buffered salt solutions without Ca<sup>++</sup> or Mg<sup>++</sup>, such as PBS, modified with 2% fetal bovine serum (FBS).

**Table 1. Optimum Number of Rat 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 <sup>10</sup>	2 x 10 <sup>9</sup> - 1.5 x 10 <sup>10</sup>
0.6"	5 x 10 <sup>8</sup>	10 <sup>8</sup> - 1.5 x 10 <sup>9</sup>
0.5"	10 <sup>8</sup>	5 x 10 <sup>7</sup> - 3 x 10 <sup>8</sup>
0.3"	5 x 10 <sup>7</sup>	2 x 10 <sup>7</sup> - 8 x 10 <sup>7</sup>
0.1"	10 <sup>6</sup> - 10 <sup>7</sup>	10 <sup>5</sup> - 2 x 10 <sup>7</sup>

## Abbreviated Procedure - Rat Cells

Refer to Manual for Further Detail.



1. Resuspend cells at  $5 \times 10^7$  cells/mL or within the acceptable range of  $2 - 8 \times 10^7$  cells/mL in recommended medium (see previous page).
2. Add 100  $\mu$ L antibody cocktail/mL cells. Mix well.
3. Incubate on ice for 30 minutes or at room temperature for 15 minutes.
4. Add 60  $\mu$ L of magnetic colloid/mL cells. Mix well.
5. Incubate on ice for 30 minutes or at room temperature for 15 minutes.
6. 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. Check for air bubbles. Place in magnet. Proceed with 6c.

**Table 2. 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.

- 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 6c.
  - \*\*Note: 0.1" column is primed quickly.
  - c) Wash (top down) with 3X column volume of recommended medium (see Table 3).
7. Load sample. Wash through with recommended medium, collecting sample volume plus 3X column volume as flowthrough (see Table 3).

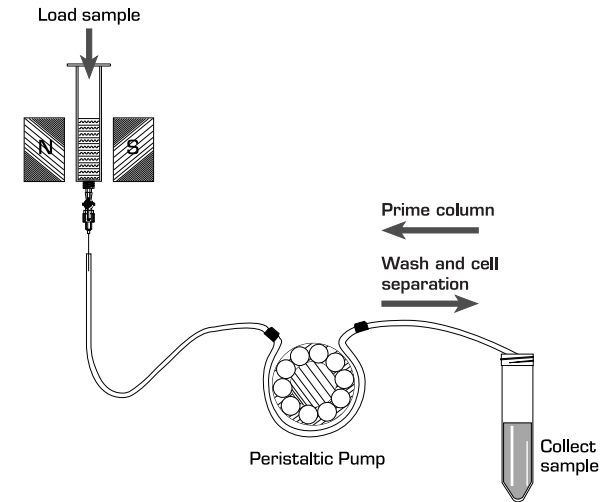
**Table 3. 3X Column Volume**

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:

