

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.
- Use DNase (Catalog #07900) at 0.1 mg/mL (final concentration) when working with previously frozen or clumpy samples. If you are running a gravity separation and the cells are clumpy, filter the cell suspension through a 70 µm mesh prior to labeling.

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.



Procedure - Rhesus Monkey Cells*



Lymphocyte (T, CD4⁺, CD8⁺) Enrichment or Depletion

Use a nucleated cell suspension from peripheral blood. This method has been designed for use with peripheral blood buffy coat cells from which erythrocytes have been removed by lysis. Alternatively, mononuclear (Ficolled) cells or leukapheresis preparations may be used.

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

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

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

*These cocktails have not been tested on cells from other non-human primate species.

Abbreviated Procedure - Rhesus Monkey Cells



Refer to Manual for Further Details.

- Resuspend cells at 5×10^7 cells/mL or within the acceptable range of $2 - 8 \times 10^7$ cells/mL in recommended medium (see previous page).
- T cell enrichment:** Add 100 μ L cocktail/mL cells.
 - CD4⁺ or CD8⁺ T cell enrichment:** Add 100 μ L of the T cell enrichment cocktail/mL cells and add 20 μ L/mL cells of the anti-CD8 or anti-CD4 TAC (tetrameric antibody complexes) supplied as a separate vial.
 - T cell depletion:** Add 20 μ L/mL cells each of the three tetrameric antibody complexes provided (anti-CD3, anti-CD4 and anti-CD8).
- Incubate on ice for 30 minutes or at room temperature for 15 minutes.
- Add 60 μ L of magnetic colloid/mL cells. Mix well.
- Incubate on ice for 30 minutes or at room temperature for 15 minutes.
- Prepare column as follows (refer to diagrams on opposite page):
 - 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 to Step 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.

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

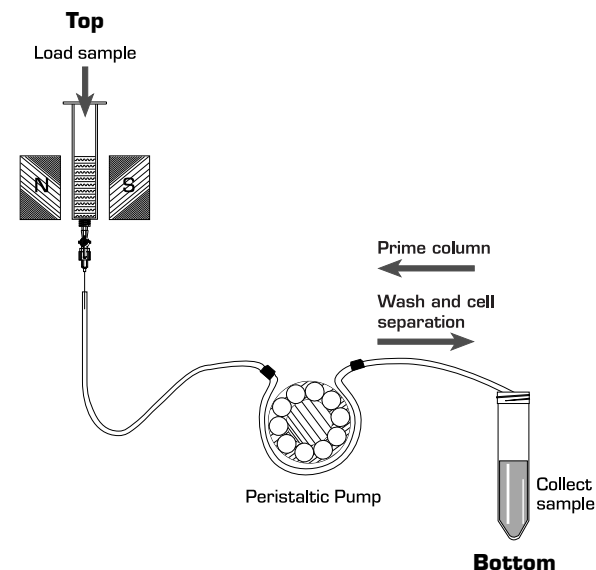
Table 3. 3X Column Volume

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:

