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1.0 Introduction

StemSep™ is an immunomagnetic column-based cell isolation system that uses antibody complexes recognizing specific cell surface antigens and magnetic particles to select or deplete cells of interest. Labeled cells are linked to StemSep™ Magnetic Colloid and are separated using a column. For positive cell isolation, cells of interest are labeled and bind to the column while unlabeled cells pass through. For negative cell isolation, unwanted cells are labeled for depletion and bind to the column while cells of interest pass through and are untouched.

To support the StemSep™ cell isolation system, STEMCELL Technologies uses a wide selection of antibodies for designing custom column-based cell separation reagents for the isolation of your desired cell type. Please contact us at techsupport@stemcell.com for more information.

1.1 StemSep™ Systems

Some items may no longer be available. Contact us at techsupport@stemcell.com for more information.

1.1.1 Columns

Columns are available in the following sizes:

- 0.1” diameter - for gravity feed only
- 0.3” diameter - for gravity feed or pump feed
- 0.5” diameter - for gravity feed or pump feed
- 0.6” diameter - for gravity feed or pump feed
- 1.0” diameter - for pump feed only

Refer to Table 2 and Table 3 for recommended cell numbers to use with each column.

1.1.2 Magnets

![Figure 1. StemSep™ Red Magnet with Stand](image)

The StemSep™ Red Magnet is designed to run one gravity feed separation with a 0.1” or 0.3” StemSep™ column. Depending on the column size and selection method, a range of $1 \times 10^5$ - $1 \times 10^8$ cells can be loaded per column. Refer to Table 2 and Table 3 for recommended cell numbers.
Figure 2. StemSep™ Green Magnet with Stand

The StemSep™ Green Magnet is designed to run one gravity feed separation with the following StemSep™ column sizes: 0.1", 0.3", 0.5", and 0.6". Depending on the column size and selection method, a range of $1 \times 10^5 - 2 \times 10^9$ cells can be loaded per column. Refer to Table 2 and Table 3 for recommended cell numbers.

Figure 3. StemSep™ Blue Magnet with Stand and Pump

The StemSep™ Blue Magnet is designed to run four simultaneous separations with the following StemSep™ column sizes: 0.3", 0.5", and 0.6". Depending on the column size and selection method, a range of $2 \times 10^7 - 2 \times 10^9$ cells can be loaded per column. This magnet and stand can accommodate only pump feed columns. Refer to Table 2 and Table 3 for recommended cell numbers.
Figure 4. StemSep™ Black Magnet with Stand and Pump

The StemSep™ Black Magnet is designed to run a single pump feed 1.0” bulk column. A range of $2 \times 10^8$ - $1.5 \times 10^{10}$ nucleated cells can be processed with this column. This magnet and stand can accommodate only pump feed columns in the following sizes: 0.3”, 0.5”, 0.6”, and 1.0”. Refer to Table 2 and Table 3 for recommended cell numbers.

1.2 Care of Magnets

After completing a cell separation, wipe the magnet clean with distilled water and then with alcohol to dry the magnet. To disinfect the magnet, wipe with 10% diluted household bleach and then wipe off with a water-dampened cloth to avoid corrosion of metal parts. Do not submerge the magnet in liquid.
2.0 Recommended Medium

Dulbecco’s Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905), EasySep™ Buffer (Catalog #20144), or phosphate-buffered saline (PBS) containing 2% FBS either with or without 1 mM EDTA. Medium should be free of Ca++ and Mg++. HBSS, Modified (Without Ca++ and Mg++; Catalog #37250) may be substituted for PBS. Human serum albumin (HSA) may be substituted for fetal bovine serum (FBS).

To optimize the recovery of adherent cells such as monocytes and dendritic cells, add EDTA to a final concentration of 1 mM to all media for cell labeling and separation. Kits for isolating monocytes and dendritic cells are available through custom orders only; contact techsupport@stemcell.com for more information.

To optimize the recovery of circulating epithelial cells, add EDTA to a final concentration of 2 mM to all media for cell labeling and separation. Kits for isolating epithelial cells are available through custom orders only; contact techsupport@stemcell.com for more information.

2.1 Density Gradient Medium

Lymphoprep™ (Catalog #07800) or other medium with a density of 1.077 g/mol.
3.0 Sample Preparation

3.1 Anticoagulant

The use of heparin as an anticoagulant is not recommended, as it may cause aggregation and a lower separation efficiency when isolating certain cell types. If samples are collected in heparin, wash twice with phosphate-buffered saline (D-PBS [Without Ca++ or Mg++]; Catalog #37350) and continue procedure using medium without heparin. Ensure that there is a sufficient concentration of anticoagulant (citrate, ACD, EDTA, etc.) in all wash media.

3.2 Human Cell Suspensions

All samples must have < 20 red blood cells (RBCs) per nucleated cell (e.g. a hematocrit of < 5%). This can be achieved with ammonium chloride lysis or density gradient centrifugation, where appropriate (Table 1).

- Progenitor and primitive progenitor cells can be enriched from mobilized peripheral blood/leukapheresis, bone marrow, or cord blood samples
- Lymphocytes (T cells, B cells, and NK cells), dendritic cells, and monocytes are designed for enrichment from peripheral blood mononuclear cell (PBMC) suspensions
- Basophils can be enriched from whole blood, mobilized peripheral blood/leukapheresis, bone marrow, or cord blood samples
- Eosinophils must be enriched from a polymorphonuclear cell (PMNC) suspension

Do not resuspend cells to a final volume of less than 100 µL, even if it means that the concentration is less than 2 x 10^7 cells/mL. The volume of the cell suspension will determine the volume of cocktail and colloid to add in section 4.1.

Table 1. Sample Source and RBC Removal Method Prior to StemSep™ Cell Separation

<table>
<thead>
<tr>
<th>SAMPLE SOURCE</th>
<th>PROGENITOR</th>
<th>LYMPHOCYTE</th>
<th>BASOPHIL</th>
<th>EOSINOPHIL</th>
<th>TUMOR ENRICHMENT</th>
<th>DEPLETION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobilized peripheral blood (leukapheresis)</td>
<td>Density gradient (or lysis if hematocrit is &gt; 5%)</td>
<td>Density gradient</td>
<td>Lysis if hematocrit is &gt; 5%</td>
<td>PMNC preparation</td>
<td>Density gradient (or lysis if hematocrit is &gt; 5%)</td>
<td>Density gradient (or lysis if hematocrit is &gt; 5%)</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>---</td>
<td>Density gradient</td>
<td>Lysis</td>
<td>PMNC preparation</td>
<td>Density gradient or lysis</td>
<td>Density gradient or lysis</td>
</tr>
<tr>
<td>Cord blood*</td>
<td>Density gradient or lysis</td>
<td>Density gradient</td>
<td>Lysis</td>
<td>PMNC preparation</td>
<td>---</td>
<td>Density gradient or lysis</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Density gradient or lysis</td>
<td>Density gradient</td>
<td>---</td>
<td>---</td>
<td>Density gradient or lysis</td>
<td>Density gradient or lysis</td>
</tr>
<tr>
<td>Bone marrow buffy coat</td>
<td>Density gradient (or lysis if hematocrit is &gt; 5%)</td>
<td>Density gradient</td>
<td>---</td>
<td>---</td>
<td>Density gradient (or lysis if hematocrit is &gt; 5%)</td>
<td>Density gradient (or lysis if hematocrit is &gt; 5%)</td>
</tr>
</tbody>
</table>

PMNC - polymorphonuclear cells

*Cord blood RBCs may be resistant to lysis. Ensure that the sample contains < 20 RBCs/nucleated cell. It may be necessary to use a density gradient medium (e.g. Lymphoprep™, Catalog #07800).
Table 2. Nucleated Cell Numbers for Human and NHP Starting Samples for Various Column Sizes

<table>
<thead>
<tr>
<th>COLUMN SIZE</th>
<th>OPTIMUM # OF CELLS</th>
<th>NEGATIVE SELECTION</th>
<th>POSITIVE SELECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RANGE OF CELL #</td>
<td>RANGE OF CELL #</td>
<td></td>
</tr>
<tr>
<td>0.1&quot;</td>
<td>1 x 10^6 - 1 x 10^7</td>
<td>1 x 10^6 - 2 x 10^7</td>
<td>1 x 10^6 - 2 x 10^7</td>
</tr>
<tr>
<td>0.3&quot;</td>
<td>5 x 10^7</td>
<td>2 x 10^7 - 8 x 10^7</td>
<td>2 x 10^7 - 1 x 10^8</td>
</tr>
<tr>
<td>0.5&quot;</td>
<td>1 x 10^8</td>
<td>5 x 10^7 - 3 x 10^8</td>
<td>5 x 10^7 - 5 x 10^8</td>
</tr>
<tr>
<td>0.6&quot;</td>
<td>5 x 10^8</td>
<td>1 x 10^8 - 1.5 x 10^9</td>
<td>1 x 10^8 - 2 x 10^9</td>
</tr>
<tr>
<td>1.0&quot;</td>
<td>1 x 10^10</td>
<td>2 x 10^9 - 1.5 x 10^10</td>
<td>2 x 10^9 - 1.5 x 10^10</td>
</tr>
</tbody>
</table>

NOTE: When using PBMCs, the column capacity may be doubled.

3.2.1 Mobilized Peripheral Blood/Leukapheresis Samples - Human

1. Centrifuge over a density gradient medium or lyse RBCs if the hematocrit is > 5% (Table 1).
2. Wash cells twice with PBS or other medium without Ca++ and Mg++.
3. Resuspend cells in recommended medium at the concentration indicated below:
   - For 0.1”, 0.3”, 0.5”, and 0.6” columns: Resuspend cells at 5 x 10^7 nucleated cells/mL
     (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable)
   - For 1.0” column: Resuspend cells at 1 x 10^8 nucleated cells/mL

3.2.2 Whole Blood, Cord Blood, and Bone Marrow Samples - Human

These cell suspensions require an ammonium chloride lysis or centrifugation over a density gradient medium (where appropriate) to remove most of the RBCs (to < 20 RBCs per nucleated cell or a hematocrit of < 5%). To lyse the RBCs, use the following procedure:
1. Centrifuge cells and remove supernatant.
2. Resuspend in Ammonium Chloride Solution (Catalog #07800) as follows:
   - For whole blood and cord blood samples, resuspend in 10 times the original volume
   - For bone marrow samples, resuspend in 5 times the original volume
3. Incubate on ice for 15 minutes.
4. Centrifuge and discard the supernatant.
5. Wash cells with recommended medium.
6. Resuspend cells in recommended medium at the concentration indicated below:
   - For 0.1”, 0.3”, 0.5”, and 0.6” columns: Resuspend cells at 5 x 10^7 nucleated cells/mL
     (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable)
   - For 1.0” column: Resuspend cells at 1 x 10^8 nucleated cells/mL

3.2.3 Buffy Coat Samples - Human

Buffy coat suspensions vary greatly in RBC content. If there are > 20 RBCs per nucleated cell, lyse using the whole blood preparation (section 3.2.2) except resuspend to 3 times the original sample volume. Otherwise, wash and resuspend using the leukapheresis preparation instructions (section 3.2.1).

3.2.4 Density Gradient Cell Preparation - Human

Treat as a leukapheresis preparation, but the column capacities (Table 2) may be doubled.
3.2.5 Polymorphonuclear Cells (PMNCs) - Human

IMPORTANT: Do not use dextran sedimentation to prepare cells.

PMNC-rich suspensions for isolation of eosinophils may be prepared by either ammonium chloride lysis or HetaSep™ RBC sedimentation, as described below.

A. Ammonium Chloride Lysis
1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Carefully perform a standard density gradient separation (e.g. Lymphoprep™; Catalog #07801). Do not use SepMate™.
3. Remove and discard the plasma layer, the band of mononuclear cells, and the density gradient medium, leaving the RBC pellet intact.
4. Add Ammonium Chloride Solution (Catalog #07800) to the RBC pellet at 10X the pellet volume. Mix well.
5. Incubate on ice for 15 minutes, then centrifuge at 300 x g for 8 minutes.
6. Discard the supernatant and wash the pellet with cold recommended medium, centrifuging at 250 x g for 10 minutes.
7. Discard supernatant and resuspend cells in recommended medium as follows:
   - For 0.1", 0.3", 0.5", and 0.6" columns: Resuspend cells at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable)
   - For 1.0" column: Resuspend cells at 1 x 10^8 nucleated cells/mL

B. HetaSep™ RBC Sedimentation
1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Follow the protocol in the HetaSep™ (Catalog #07906) Product Information Sheet (Document #29622).
3. After the final centrifugation step, discard supernatant and resuspend cells in recommended medium as follows:
   - For 0.1", 0.3", 0.5", and 0.6" columns: Resuspend cells at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable)
   - For 1.0" column: Resuspend cells at 1 x 10^8 nucleated cells/mL

3.2.6 Previously Frozen or Aggregated Samples - Human

Use established procedures for thawing cells. When resuspending thawed cells, concentrated DNase I Solution (1 mg/mL; Catalog #07900) may be added directly to the cell pellet at 250 µL/mL of cell pellet. Top up sample with recommended medium without EDTA. Do not vortex sample; instead, mix with a pipette or by hand agitation. Incubate at room temperature (15 - 25°C) for 15 minutes.

Resuspend thawed cells in recommended medium without EDTA, containing DNase I Solution (0.1 mg/mL final concentration), as follows:
   - For 0.1", 0.3", 0.5", and 0.6" columns: Resuspend cells at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable)
   - For 1.0" column: Resuspend cells at 1 x 10^8 nucleated cells/mL

Note: For gravity feed separations of excessively aggregated cell suspensions, filter suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216).
3.3 Non-Human Primate (NHP) Cell Suspensions

StemSep™ cocktails for enriching NHP T lymphocytes (T cells, CD4+ and CD8+ T cells) or for depleting T lymphocytes are designed for peripheral blood buffy coat suspensions that have had RBCs removed by lysis. The cocktails can also be used with PBMC suspensions or with mobilized peripheral blood/leukapheresis preparations.

3.3.1 Buffy Coat Samples - NHP

These cell suspensions require an ammonium chloride lysis to remove the RBCs, as described below. 

*Note: Residual RBCs will not be depleted by the procedure and will be recovered in the column flow-through/enriched cell fraction.*

1. Centrifuge cells and remove supernatant.
2. Resuspend in Ammonium Chloride Solution (Catalog #07800) at 3 w 4 times the original volume.
3. Incubate on ice for 15 minutes.
4. Centrifuge and discard the supernatant.
5. Wash cells with recommended medium.
6. Resuspend cells in recommended medium as follows:
   - For 0.1”, 0.3”, 0.5”, and 0.6” columns: Resuspend cells at 5 x 10^7 nucleated cells/mL (a range of 2 w 8 x 10^7 nucleated cells/mL is acceptable)
   - For 1.0” column: Resuspend cells at 1 x 10^8 nucleated cells/mL

3.3.2 Mononuclear Cell Samples - NHP

Mononuclear cell suspensions may still contain RBCs. Removal of the residual RBCs is recommended with a lysis step. Proceed as described in section 3.3.1 for buffy coat samples.

3.3.3 Density Gradient Cell Preparation - NHP

Samples may be prepared with density gradient centrifugation, as follows:

1. Centrifuge diluted sample over a density gradient medium.
2. Wash cells twice with PBS or other medium without Ca++ and Mg++.

*Note: For cynomolgus samples, centrifuge over density gradient medium that has been diluted to 90% with PBS.*

3.3.4 Previously Frozen or Aggregated Samples - NHP

Use established procedures for thawing cells. When resuspending thawed cells, concentrated DNase I Solution (1 mg/mL; Catalog #07900) may be added directly to the cell pellet at 250 µL/mL of cell pellet. Top up sample with recommended medium without EDTA. Do not vortex sample; instead, mix with a pipette or by hand agitation. Incubate at room temperature (15 - 25°C) for 15 minutes.

Resuspend thawed cells in recommended medium without EDTA, containing DNase I Solution (0.1 mg/mL final concentration), at a concentration of 5 x 10^7 nucleated cells/mL.

*Note: For gravity feed separations of excessively aggregated cell suspensions, filter suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216).*
3.4 Mouse Cell Suspensions

Mouse hematopoietic progenitor cells are typically isolated from bone marrow, and leukocyte sub-populations are typically isolated from spleen cell suspensions.

The normal rat serum used in cell preparation blocks Fc receptors on mouse cells, preventing the non-specific binding of the rat antibody. Addition of normal rat serum to the sample prior to labeling is required for optimal cell recovery in mouse separations. Normal rat serum (provided when required for the kit) is usually added at a concentration of 50 µL/mL of sample prior to isolation, as indicated in the protocol in Table 6.

Do not resuspend cells to a final volume of less than 200 µL, even if it means that the concentration is less than 2 x 10^7 cells/mL. The volume of the cell suspension will determine the volume of cocktail and colloid to add in section 4.1.

Table 3. Nucleated Cell Numbers for Mouse and Rat Starting Samples for Various Column Sizes

<table>
<thead>
<tr>
<th>COLUMN SIZE</th>
<th>OPTIMUM # OF CELLS</th>
<th>RANGE OF CELL #</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1&quot;</td>
<td>1 x 10^6 - 1 x 10^7</td>
<td>1 x 10^5 - 2 x 10^7</td>
</tr>
<tr>
<td>0.3&quot;</td>
<td>5 x 10^6</td>
<td>2 x 10^5 - 8 x 10^6</td>
</tr>
<tr>
<td>0.5&quot;</td>
<td>1 x 10^8</td>
<td>5 x 10^7 - 4 x 10^8</td>
</tr>
<tr>
<td>0.6&quot;</td>
<td>5 x 10^8</td>
<td>1 x 10^9 - 1.5 x 10^9</td>
</tr>
<tr>
<td>1.0&quot;</td>
<td>1 x 10^10</td>
<td>2 x 10^8 - 1.5 x 10^10</td>
</tr>
</tbody>
</table>

3.4.1 Bone Marrow - Mouse

1. Flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23 gauge needle. Disperse clumps by gently passing the cell suspension through the syringe several times.

   OR

   Crush bones using a mortar and pestle.

2. Remove remaining clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216).

3. Centrifuge at 120 x g for 10 minutes and discard supernatant.

4. Resuspend at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable) in recommended medium.

3.4.2 Spleen - Mouse

1. Disrupt the spleen in recommended medium.

2. Remove cell aggregates by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216).

3. Centrifuge at 120 x g for 10 minutes and discard supernatant.

4. Resuspend at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable) in recommended medium.

Note: Ammonium chloride treatment is not recommended when preparing the cells for separation.
3.4.3 Previously Frozen or Aggregated Samples - Mouse

Use established procedures for thawing cells. When resuspending thawed cells, concentrated DNase I Solution (1 mg/mL; Catalog #07900) may be added directly to the cell pellet at 250 µL/mL of cell pellet. Top up sample with recommended medium without EDTA. Do not vortex sample; instead, mix with a pipette or by hand agitation. Incubate at room temperature (15 - 25°C) for 15 minutes. Resuspend thawed cells in recommended medium without EDTA, containing DNase I Solution (0.1 mg/mL final concentration), at a concentration of 5 x 10^7 nucleated cells/mL. Note: For gravity feed separations of excessively aggregated cell suspensions, filter suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216).

3.5 Rat Cell Suspensions

Rat lymphocytes are typically isolated from spleen cell suspensions.

3.5.1 Spleen - Rat

Note: Ammonium chloride treatment is not recommended when preparing rat spleen cells for separation.

1. Disrupt spleen in recommended medium.
2. Remove cell aggregates by passing cell suspension through a pre-wetted 70 µm mesh nylon strainer (e.g. Catalog #27216).
3. Centrifuge at 120 x g for 10 minutes with the brake off. Discard supernatant.
4. Resuspend at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable) in recommended medium.
5. If aggregation persists, pass the cell suspension through a pre-wetted 70 µm mesh nylon strainer a second time and centrifuge again at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend at 5 x 10^7 nucleated cells/mL (a range of 2 - 8 x 10^7 nucleated cells/mL is acceptable) in recommended medium. Note: Do not resuspend cells to a final volume of less than 200 µL, even if it means that the concentration is less than 2 x 10^7 cells/mL. The volume of the cell suspension will determine the volume of cocktail and colloid to add in section 4.1.

3.5.2 Previously Frozen or Aggregated Samples - Rat

Use established procedures for thawing cells. When resuspending thawed cells, concentrated DNase I Solution (1 mg/mL; Catalog #07900) may be added directly to the cell pellet at 250 µL/mL of cell pellet. Top up sample with recommended medium without EDTA. Do not vortex sample; instead, mix with a pipette or by hand agitation. Incubate at room temperature (15 - 25°C) for 15 minutes. Resuspend thawed cells in recommended medium without EDTA, containing DNase I Solution (0.1 mg/mL final concentration), at a concentration of 5 x 10^7 nucleated cells/mL. Do not resuspend cells to a final volume of less than 100 µL, even if it means that the concentration is less than 2 x 10^7 cells/mL. The volume of the cell suspension will determine the volume of cocktail and colloid to add in section 4.1. Note: For gravity feed separations of excessively aggregated cell suspensions, filter suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216).
4.0 Antibody Labeling and StemSep™ Procedures

4.1 Antibody Labeling
See Recommended Medium (section 2.0) and Sample Preparation (section 3.0) for more information.

Table 4. StemSep™ Antibody Labeling Procedure for Human Samples

<table>
<thead>
<tr>
<th>STEP</th>
<th>INSTRUCTION</th>
<th>HUMAN SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepare sample (section 3.2) at the indicated cell concentration in recommended medium (section 2.0).</td>
<td>5 x 10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL</td>
</tr>
<tr>
<td></td>
<td>Optional</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For monocyte and dendritic cell enrichment, add anti-CD32 (FcγRII) to sample. Mix and incubate.</td>
<td>On ice for 10 minutes</td>
</tr>
<tr>
<td></td>
<td>Optional</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For custom cocktails that contain biotinylated antibodies, add biotinylated antibody. Mix and incubate.</td>
<td>See vial label for volume</td>
</tr>
<tr>
<td>2</td>
<td>Add cocktail to sample. Mix and incubate.</td>
<td>• On ice for 30 minutes OR • RT for 15 minutes</td>
</tr>
<tr>
<td>3</td>
<td>Add magnetic colloid to sample. Mix and incubate.</td>
<td>• On ice for 30 minutes OR • RT for 15 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Prepare column and load samples. See section 4.2 for gravity feed or section 4.3 for pump feed instructions</td>
<td></td>
</tr>
</tbody>
</table>

RT - room temperature
### Table 5. StemSep™ Antibody Labeling Procedure for NHP and Rat Samples

<table>
<thead>
<tr>
<th>STEP</th>
<th>INSTRUCTION</th>
<th>RAT OR NHP SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepare sample (sections 3.3 and 3.5) at the indicated cell concentration in recommended medium (section 2.0).</td>
<td>5 x 10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL</td>
</tr>
<tr>
<td>2</td>
<td>Add cocktail to sample.</td>
<td>See vial label or corresponding PIS for volume</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate.</td>
<td>• On ice for 30 minutes OR • RT for 15 minutes</td>
</tr>
<tr>
<td>3</td>
<td>Add magnetic colloid to sample.</td>
<td>60 µL/mL of sample</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate.</td>
<td>• On ice for 30 minutes OR • RT for 15 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Prepare column and load samples.</td>
<td>See section 4.2 for gravity feed or section 4.3 for pump feed instructions</td>
</tr>
</tbody>
</table>

RT - room temperature

### Table 6. StemSep™ Antibody Labeling Procedure for Mouse Samples

<table>
<thead>
<tr>
<th>STEP</th>
<th>INSTRUCTION</th>
<th>MOUSE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepare sample (section 3.4) at the indicated cell concentration in recommended medium (section 2.0).</td>
<td>5 x 10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL</td>
</tr>
<tr>
<td>2</td>
<td>Add rat serum to sample.</td>
<td>50 µL/mL of sample</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate.</td>
<td>2 - 8°C for 15 minutes</td>
</tr>
<tr>
<td>3</td>
<td>Add cocktail to sample.</td>
<td>See vial label or corresponding PIS for volume</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate.</td>
<td>2 - 8°C for 15 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Wash and resuspend cells in original volume.</td>
<td>Use same volume as step 1</td>
</tr>
<tr>
<td>5</td>
<td>Add Anti-Biotin TAC.</td>
<td>100 µL/mL of sample</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate.</td>
<td>2 - 8°C for 15 minutes</td>
</tr>
<tr>
<td>6</td>
<td>Add magnetic colloid to sample.</td>
<td>60 µL/mL of sample</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate.</td>
<td>2 - 8°C for 15 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Prepare column and load samples.</td>
<td>See section 4.2 for gravity feed or section 4.3 for pump feed instructions</td>
</tr>
</tbody>
</table>

TAC - tetrameric antibody complex
4.2  Separation Procedure - Gravity Feed

Prepare the column(s) while the cells are incubating with the antibody cocktail.

4.2.1 StemSep™ Column Assembly - Gravity Feed

Figure 5. StemSep™ Column Assembly for Gravity Feed Separations

1. Place the magnet in the stand.
2. Remove the StemSep™ column from its sterile package without touching the luer fitting. The 0.1” column comes with an extender. This is required for use with the green magnet and red magnets purchased before January 1999. Aseptically attach extender if required.
3. Place the column in the magnet. Be careful not to touch any surface with the tip of the luer lock.
   Note: For 0.1” columns being used with a green magnet, the whole column will sit in the gap of the magnet with the column rim resting on the top of the magnet.
   CAUTION: The magnet will grab the column.
4. Remove 3-way stopcock from its sterile package and aseptically attach to the column or column extender (0.1” column only).
5. Remove the blunt-end needle from its sterile package, keeping the cover on the end of the needle until section 4.2.2 step 7 (washing the column), and aseptically attach the hub to luer fitting on the 3-way stopcock directly below the column (23 gauge blunt-end needle for 0.1”, 0.3” and 0.5” columns; 22 gauge blunt-end needle for 0.6” columns).
6. Check all connections to ensure they do not leak.
4.2.2 Column Preparation - Gravity Feed

**IMPORTANT:** Do not let the column run dry at any time while priming, washing, or loading the column.

1. Assemble the magnet and column as described in section 4.2.1.
2. Remove the gauze from the top of the column (0.3", 0.5", and 0.6" columns only).
3. Set 3-way stopcock to allow flow from the side connection into column.
4. Fill a sterile syringe (included in the kit) with PBS (without FBS or other protein), remove air bubbles, and attach to the side connection of 3-way stopcock (Figure 6).
5. For 0.1" column:
   - Rapidly depress plunger of syringe with firm even pressure to deliver PBS up the column. Deliver the entire 1 mL of PBS. Remove any air bubbles trapped in the column matrix by gently moving the plunger of the side syringe in and out. Repeat 5 - 10 times, making sure that the level of PBS does not fall below the top of the column matrix (more PBS may be added to the top if needed). End the priming by pulling back 500 µL of PBS and any air bubbles into the side syringe.
6. For 0.3", 0.5", and 0.6" columns:
   - Slowly depress plunger of syringe to deliver PBS up the column until the level of PBS is above the mesh matrix of the column. Do not allow air bubbles to enter the column matrix. If there are air bubbles in the column matrix, they may be dislodged by sharply tapping the side of the column. If there is an air bubble lodged in the 3-way stopcock, add PBS to the top of the column and then pull the bubble out into the side syringe. If the bubble cannot be removed, proceed; it will likely remain in the 3-way stopcock and not interfere with separation.

   If the column stops running at any point, introduce a small volume of PBS from the side syringe into the column. This should remove any air bubbles trapped at the top of the column which can disrupt the flow. An air lock in the needle may be removed by redirecting the flow of buffer from the side syringe out through the needle. The 0.1" column will not run dry, but for best performance the separation procedure should not be prolonged unnecessarily.

7. To wash the column, place a waste container below the blunt-end needle, remove the needle cover, and add recommended medium to the top of the column. Turn the 3-way stopcock so the flow is from the column down through the needle (as shown in Figure 6; i.e. side exit is closed).
8. Continue adding recommended medium until three column volumes have been collected, as follows:
   - 0.1" column - collect 1 mL
   - 0.3" column - collect 8 mL
   - 0.5" column - collect 15 mL
   - 0.6" column - collect 25 mL

   **IMPORTANT:** Do not let the column run dry at any time.

9. Turn the 3-way stopcock to stop flow of medium from the column when the fluid level is just above the column matrix. The column is now ready for the separation procedure.
4.2.3 Separation - Gravity Feed

**IMPORTANT:** Do not let the column run dry at any time during the priming, washing, or loading of the column.

1. Load the sample into the top of the column.
2. Turn the 3-way stopcock to start the flow of medium down through the needle into the collection tube. Allow the sample to run into the column matrix.
3. Add recommended medium to top of column until three column volumes plus the volume of the start sample have been collected, as follows:
   - 0.1” column - collect 1.5 mL
   - 0.3” column - collect 8 mL
   - 0.5” column - collect 15 mL
   - 0.6” column - collect 25 mL
   **IMPORTANT:** Do not let the column run dry at any time.
4. Turn the 3-way stopcock to stop the flow of medium.
   *Note: This collected flow-through fraction contains the enriched cells for the cell enrichment cocktails or the purged cells for the purging cocktails.*
5. Let remaining fluid drain from the column into a waste container.
6. Disconnect the 3-way stopcock.
7. Remove the column from the top of magnet.
8. Discard used column, syringe, 3-way stopcock, etc. into an appropriate waste container.

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**Figure 6. Separation Diagram for Gravity Feed Separations**
4.2.4 Removing Cells From the Column - Gravity Feed

Select the appropriate protocol for removing cells from the StemSep™ column based on the type of magnet and column size being used.

**Magnets Without Keyhole Gaps**

1. Run the column as described in section 4.2.3 steps 1 - 2, stopping when the fluid level is just above the column matrix.
2. Ensure the side syringe is empty. If necessary, turn the 3-way stopcock such that the column is “off” (flow permitted between side syringe and needle) and expel medium from the side syringe out of the needle into a waste container.
3. Carefully remove the column from the magnet. The strong magnetic field may require you to steady the magnet while gently pulling the column from the magnet gap.
4. Remove the cells from the column as follows:
   a) Turn the 3-way stopcock so that the needle is “off” (flow permitted between side syringe and column).
   b) Add medium to the top of the column as follows:
      - 0.1” column - add 0.5 mL
      - 0.3” column - add 1 mL
   c) Flush medium back and forth between the column and side syringe several times. Draw all medium into the side syringe.
   d) Turn the 3-way stopcock so that the side syringe is “off”.
   e) Disconnect the side syringe and expel all medium and positively selected cells into a collection tube.
5. Reconnect the side syringe and repeat step 4 for maximum recovery of cells from the column.
6. Discard the used column, pump tubing, etc. into an appropriate waste container.

**Magnets With Keyhole Gaps**

1. Run the column as described in section 4.2.3 steps 1 - 2, stopping when the fluid level is just above the column matrix.
2. Empty the side syringe by turning the 3-way stopcock such that the column is “off” (flow permitted between side syringe and needle) and expelling the medium from the side syringe out of the needle into a waste container.
3. Place a new collection tube under the needle.
4. Turn the 3-way stopcock such that the needle is “off” (flow permitted between side syringe and column) and draw all medium from column into the side syringe.
5. Turn the 3-way stopcock such that the column is “off” (flow permitted between side syringe and needle) and expel medium from side syringe into new collection tube.
6. Remove the column from the magnet by disconnecting the column from the 3-way stopcock and pulling the column vertically from the magnet gap. Immediately reconnect the column to the 3-way stopcock.
7. Remove cells from the column as follows:
   a) Remove the side syringe and fill with medium as follows:
      - 0.1” column - add 0.8 mL
      - 0.3” column - add 2 mL
• 0.5” column - add 4 mL
• 0.6” column - add 6 mL

b) Reattach the side syringe and turn the 3-way stopcock so that the needle is “off” (flow permitted between side syringe and column). Flush medium back and forth through the column several times and draw the entire volume back into the side syringe.

c) Turn the 3-way stopcock so that the side syringe is “off”.

d) Remove the side syringe and expel the medium into the collection tube.

8. Repeat step 7 for maximum recovery of cells from the column.

9. Discard the used column, pump tubing, etc. into an appropriate waste container.

4.3 Separation Procedure - Pump Feed

Prepare the column(s) while the cells are incubating with the antibody cocktail.

4.3.1 StemSep™ Column Assembly - Pump Feed

Figure 7. StemSep™ Column Assembly for Pump Feed Separations

1. Remove the StemSep™ column from its sterile package without touching the luer fitting.
2. Remove the StemSep™ pump tubing from its sterile package.
3. Aseptically attach the hub to the luer fitting on column.
4. Check all connections to ensure they do not leak.

4.3.2 Column Preparation - Pump Feed

IMPORTANT: Do not let the column run dry at any time while priming, washing, or loading the column.

1. Place the magnet in the stand.
2. Assemble the column as described in section 4.3.1 and place in the magnet by holding the magnet and slowly lowering the column down into the gap. Do not insert the column from the front.

*Note*: If one or two columns are run alone in the blue magnet, use the column support supplied. The long portion of the support fits into the gap of the magnet behind the column(s), keeping the column(s) upright.

**CAUTION**: The magnet will grab the column.

3. Insert the pump tubing into the peristaltic pump by hooking the colored stoppers on the tubing into the grooves at the front of the peristaltic pump and running the tubing around the back of the rollers. Ensure tubing is lined up in the slot and both stoppers are connected. Connect clamps on the back of pump with a “click.” The black screws on the clamps set the calibration of the pump; do not adjust them unless calibrating the pump (refer to pump manual for detailed pump operating instructions).

4. Remove the gauze from the top of the column.

5. Remove the paper cover from the 10 cm metal end (probe) of the pump tubing. Place into sterile PBS *(without* FBS or other protein).

6. Prime the column by running the pump so the column fills from bottom to top (upwards). See Table 7 for pump settings. Do not disturb the column while priming.

7. When the level of PBS is above the column matrix, stop the pump.

8. Carefully examine the column (it may need to be removed from the magnet). If there are air bubbles in the column matrix, they may be dislodged by sharply tapping the side of the column. If a large amount of air (0.5 - 1 mL bubble) remains in the column, discard the column and prime a new column. Replace the column in the magnet.

9. Wash the column as follows:
   a) Increase the pump speed (Table 7) and reverse the pump direction to draw liquid down through the column (downwards).
   b) Add recommended medium to the top of the column until three column volumes have been collected, as follows:
      - 0.3” column - collect 8 mL
      - 0.5” column - collect 15 mL
      - 0.6” column - collect 25 mL
      - 1.0” column - collect 90 mL

**IMPORTANT**: Do not let the column run dry at any time.

10. Stop the pump when the fluid level is just above the column matrix and transfer the end probe to a collection tube. The column is now ready for the separation procedure.

### Table 7. Flow Rates and Pump Settings

<table>
<thead>
<tr>
<th>COLUMN SIZE</th>
<th>PRIMING</th>
<th>LOADING SAMPLE AND WASHING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL/min</td>
<td>PUMP SETTING</td>
</tr>
<tr>
<td>1.0”</td>
<td>2.0</td>
<td>10.0</td>
</tr>
<tr>
<td>0.6”</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>0.5”</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>0.3”</td>
<td>0.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**NOTE**: Pump setting is for the 4-channel peristaltic pump supplied by STEMCELL Technologies Inc. only.
4.3.3 Separation - Pump Feed

**LOAD SAMPLE**

Figure 8. Separation Diagram for Pump Feed Separations

**IMPORTANT:** Do not let the column run dry at any time during priming, washing, or loading the column.

1. Load the sample into the top of the column.
2. Start the pump in a downward direction and allow the sample to run into the column matrix.
3. Add recommended medium to the top of the column until three column volumes plus the volume of the start sample have been collected, as indicated below. Do not allow the top of the fluid level to reach the column matrix.
   - 0.3” column - collect 8 mL
   - 0.5” column - collect 15 mL
   - 0.6” column - collect 25 mL
   - 1.0” column - collect 90 mL

**IMPORTANT:** Do not let the column run dry at any time.

4. Stop the pump. The collected flow-through fraction is the enriched cell fraction for the cell enrichment cocktails or the purified cell fraction for the purging cocktails.
5. Pump all remaining fluid from the column into a waste container.
6. Remove the used column from the top of the magnet.
7. Discard the used column, pump tubing, etc. into a biohazardous waste container.
4.3.4 Removing Cells From the Column - Pump Feed

For 0.3”, 0.5”, and 0.6” Columns With Blue Magnet

1. Assemble column as described in section 4.3.1, but insert a 3-way stopcock between the column and the pump tubing. Ensure that the side port of the 3-way stopcock is “off” (flow permitted between the column and the pump tubing).

2. Prepare the column as described in section 4.3.2. When placing column in the magnet (step 2), carefully hold column at the top and below the 3-way stopcock and place into magnet from the front, ensuring clearance for the 3-way stopcock.

   CAUTION: Do not block the path of the column into the magnet gap; the strong magnetic field will pull the column into position in the magnet gap extremely quickly.

3. Run the column as described in section 4.3.3 steps 1 - 3, stopping when the fluid level is just above column matrix.

4. Carefully remove column from the magnet. The strong magnetic field may require you to steady the magnet while gently pulling the column from the magnet gap. Leave pump tubing in pump.

5. Attach sterile side syringe (0.1” or 0.3” columns use 3 cc; 0.5” or 0.6” columns use 10 cc or larger) to the side port of the 3-way stopcock.

6. Remove cells from the column as follows:
   a) Turn the 3-way stopcock so that the pump tubing is “off” (flow permitted between side syringe and column).
   b) Add medium to the top of the column as follows:
      • 0.1” column - add 1 mL
      • 0.3” column - add 1.5 mL
      • 0.5” column - add 3 mL
      • 0.6” column - add 4 mL
   c) Flush medium back and forth between column and side syringe several times. Draw all medium into side syringe.
   d) Turn the 3-way stopcock so that the side syringe is “off” (to prevent drips through side port of the 3-way stopcock).
   e) Disconnect side syringe and expel all medium with positively selected cells into collection tube.

7. Reconnect side syringe and repeat step 6 for maximum recovery of cells from the column.

8. Discard the used column, pump tubing, etc. into an appropriate waste container.

For 1.0” Columns With Black Magnet

1. Assemble column as described in section 4.3.1, but insert a 3-way stopcock between the column and the pump tubing. Ensure that the side port of the 3-way stopcock is “off” (flow permitted between the column and the pump tubing).

2. Prepare the column as described in section 4.3.2 steps 1 - 8, but prime column outside of the magnet by placing it in front of the magnet gap.

3. Place the column in the magnet as follows:
   a) Turn the 3-way stopcock so that the column is “off” (flow permitted between side port and pump tubing).
   b) Carefully hold column at the top with side port of the 3-way stopcock facing forward into the magnet gap. The strong magnetic field will rapidly pull the column into position.
c) Push the column from the top so that the 3-way stopcock is accessible from below the magnet.

d) Turn the 3-way stopcock so that side port is "off" (flow permitted between column and pump tubing).

4. Wash the column as described in section 4.3.2 step 9.

5. Run the column as described in section 4.3.3 steps 1 - 3.

6. Attach a 60 cc sterile syringe to side port of the 3-way stopcock and turn the 3-way stopcock so that pump tubing is "off" (flow permitted from column to side syringe). Carefully remove column and assembly from magnet, with side syringe pointing out of magnet gap (if the 3-way stopcock points straight down it will fit through the magnet). The strong magnetic field may require you to steady the magnet while gently pulling the column from the magnet gap.

7. Remove cells from the column as follows:
   a) Add 20 mL medium to the top of the column. Flush medium back and forth between column and side syringe several times. Draw all medium into side syringe.
   b) Turn the 3-way stopcock so that the side syringe is "off" (to minimize drips through side port of the 3-way stopcock).
   c) Disconnect side syringe and expel all medium with positively selected cells into collection tube.

8. Reconnect the side syringe and repeat step 7 for maximum recovery of cells from the column.

9. Discard the used column, pump tubing, etc. into an appropriate waste container.
5.0 Troubleshooting

5.1 Cell Separation
A few helpful hints and reminders to ensure a successful cell separation:

- Use the correct column size for the number of cells in the sample (see Table 2 and Table 3)
- Load the appropriate number of cells into the column (see Table 2 and Table 3)
- During the separation, place the column in the gap of the magnet and not on the side of the magnet
- Use the correct pump speed (see Table 7) and gauge of blunt-end needle (section 4.2.1)

5.2 Purity
Poor antibody binding to unwanted cells reduces the purity of the recovered cells. This could result, for example, from incubating the cell sample with the antibody and tetrameric antibody complexes at the wrong temperature and/or time. Some additional reasons purity may be decreased include:

- An excess of RBCs in the prepared sample
- Overloading the column
- Column is not washed or primed correctly, air bubbles are lodged in the column, or the column is allowed to run dry at any time
- Using antibodies that are too dilute or using too little magnetic colloid during negative selection
  OR
  Using antibodies that are too concentrated or using too much magnetic colloid during positive selection
- Collecting the cells that ran through the column, instead of those that adhered to the column

5.3 Recovery
Reduced recovery, or yield, of target cells may be reduced due to any of the following:

- Incorrect cell concentration
- Cells are not in a single-cell suspension (i.e. they are aggregated or “clumpy”)
- Overloading the column
- For mouse cell separations, not blocking cells with normal rat serum prior to the addition of antibody
- Column is not washed or primed correctly, air bubbles are lodged in the column, or the column is allowed to run dry at any time
- Collecting the cells that ran through the column, instead of those that adhered to the column
- Poor recovery of monocytes, dendritic cells, or epithelial tumor cells may result if EDTA is not added to the recommended medium