Description

Deplete red blood cells (RBCs) directly from human buffy coat by immunomagnetic negative selection. This kit can also be used to deplete RBCs from other sample types (see Table 1).

The benefits of this kit include:
- 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation or lysis
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets RBCs for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. RBC-depleted nucleated cells are simply collected into a new tube and are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

- This is the Product Information Sheet (PIS) for depleting RBCs from buffy coat. If depleting RBCs from other sample types, refer to the applicable PIS Document Number (see Table 1).

Table 1. Applicable Document Number for Other Sample Types

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>DOCUMENT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>10000003345</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>10000005629</td>
</tr>
<tr>
<td>Cord blood</td>
<td>10000005630</td>
</tr>
<tr>
<td>Leukapheresis</td>
<td>10000005627</td>
</tr>
</tbody>
</table>

Component Descriptions

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated below.

**EasySep™ RBC Depletion Reagent (Catalog #18170)**

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>COMPONENT #</th>
<th>QUANTITY</th>
<th>STORAGE</th>
<th>SHELF LIFE</th>
<th>FORMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasySep™ RBC Depletion</td>
<td>18170</td>
<td>10 mL</td>
<td>Store at 2 - 8°C.</td>
<td>Stable until expiry date (EXP) on label.</td>
<td>A suspension of magnetic particles and monoclonal antibodies in PBS.</td>
</tr>
</tbody>
</table>

**EasySep™ RBC Depletion Reagent for RoboSep™ (Catalog #18170RF)**

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>COMPONENT #</th>
<th>QUANTITY</th>
<th>STORAGE</th>
<th>SHELF LIFE</th>
<th>FORMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasySep™ RBC Depletion</td>
<td>18170C</td>
<td>4 x 2.5 mL</td>
<td>Store at 2 - 8°C.</td>
<td>Stable until expiry date (EXP) on label.</td>
<td>A suspension of magnetic particles and monoclonal antibodies in PBS.</td>
</tr>
</tbody>
</table>

Sample Preparation

**BUFFY COAT**

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer buffy coat to the required tube.

To avoid loss of monocytes, EDTA must be added to the buffy coat sample to a final concentration of 6 mM prior to labeling and separation (see step 2, Tables 2 - 4).

**NOTE:** An EDTA stock solution greater than 0.05 M is recommended to avoid over diluting the start sample.

**Recommended Medium**

PBS containing 2% fetal bovine serum (FBS). Medium should be free of Ca++ and Mg++.
### Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 2 and 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

<table>
<thead>
<tr>
<th>Table 2. EasySep™ RBC Depletion Reagent Protocol for BUFFY COAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STEP</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<tr>
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<td>9</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
</tbody>
</table>

RT - room temperature (15 - 25°C)

* Following the first magnetic separation, the collected cells may contain a significant amount of RBCs and may look similar to the original human buffy coat sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).
### Table 3. EasySep™ RBC Depletion Reagent Protocol for BUFFY COAT

<table>
<thead>
<tr>
<th>STEP</th>
<th>INSTRUCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Collect sample within the volume range. 0.5 - 1 mL 1 - 5 mL 5 - 20 mL</td>
</tr>
<tr>
<td>2</td>
<td>Add sample to required tube. 5 ml (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) 14 ml (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) 50 ml (30 x 115 mm) conical tube (e.g. Catalog #38010)</td>
</tr>
<tr>
<td>3</td>
<td>Add EDTA to sample. At a final concentration of 6 mM EDTA</td>
</tr>
<tr>
<td>4</td>
<td>Dilute sample with recommended medium. Equal volume to sample</td>
</tr>
<tr>
<td>5</td>
<td>Vortex Depletion Reagent. NOTE: Reagent should appear evenly dispersed. 30 seconds 30 seconds 30 seconds</td>
</tr>
<tr>
<td>6</td>
<td>Place the tube (without lid) into the magnet and incubate. RT for 5 minutes RT for 5 minutes RT for 10 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Carefully pipette*** (do not pour) the cell suspension into a new tube. Use a new 5 mL tube Use a new 14 mL tube Use a new 50 mL tube</td>
</tr>
<tr>
<td>8</td>
<td>Add Depletion Reagent to the new tube containing the depleted cells and mix. Use same volume as in step 5 Use same volume as in step 5 Use same volume as in step 5</td>
</tr>
<tr>
<td>9</td>
<td>Remove the tube from the magnet and place the tube from step 8 (without lid) into the magnet and incubate for a second separation. RT for 5 minutes RT for 5 minutes RT for 10 minutes</td>
</tr>
<tr>
<td>10</td>
<td>Carefully pipette*** (do not pour) the cell suspension into a new tube. Use a new 5 mL tube Use a new 14 mL tube Use a new 50 mL tube</td>
</tr>
<tr>
<td>11</td>
<td>Remove the tube from the magnet and place the tube from step 10 (without lid) into the magnet and incubate for a third separation. RT for 5 minutes RT for 5 minutes RT for 10 minutes</td>
</tr>
<tr>
<td>12</td>
<td>Carefully pipette*** (do not pour) the cell suspension into a new tube. Isolated cells are ready for use Isolated cells are ready for use Isolated cells are ready for use</td>
</tr>
</tbody>
</table>

**RT** - room temperature (15 - 25°C)

***Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).
Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 4 for detailed instructions regarding the RoboSep™ procedure.

NOTE: If using RoboSep™-S, ensure the software is at least v.1.2.0.2 and a carousel compatible with this product is installed. Contact us at techsupport@stemcell.com for more information.

Table 4. RoboSep™ RBC Depletion Reagent Protocol for BUFFY COAT

<table>
<thead>
<tr>
<th>STEP</th>
<th>INSTRUCTIONS</th>
<th>RoboSep™ (Catalog #21000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepare sample within the volume range. 2 - 5 mL</td>
<td>14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)</td>
</tr>
<tr>
<td></td>
<td>Add sample to required tube.</td>
<td>At a final concentration of 6 mM EDTA</td>
</tr>
<tr>
<td>2</td>
<td>Add EDTA to sample.</td>
<td>Human RBC Depletion 18170 - BC</td>
</tr>
<tr>
<td>3</td>
<td>Select protocol.</td>
<td>30 seconds</td>
</tr>
<tr>
<td>4</td>
<td>Vortex Depletion Reagent. NOTE: Reagent should appear evenly dispersed. Follow on-screen prompts</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Load the carousel.</td>
<td>Press the green “Run” button</td>
</tr>
<tr>
<td>6</td>
<td>Start the protocol.</td>
<td>Isolated cells are ready for use</td>
</tr>
<tr>
<td></td>
<td>Unload the carousel when the run is complete.</td>
<td></td>
</tr>
</tbody>
</table>

Notes and Tips

Not suitable for use with downstream magnetic positive selection products.

ASSESSING PURITY

For purity assessment of residual RBCs by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD235ab (Glycophorin A/B), Clone HIR2 (Catalog #60111)

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