1. General Information

Kit Contents:

<table>
<thead>
<tr>
<th>Component Name</th>
<th>Quantity</th>
<th>Format</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin Mouse Anti-Human CD3 Antibody</td>
<td>2 x 0.5mL Vial</td>
<td>Isotype: Mouse BALB/c IgG1, κ Buffer solution containing BSA and ≤0.09% sodium azide</td>
<td>Store 2–8°C DO NOT FREEZE</td>
</tr>
<tr>
<td>X-BACS™ Reagent</td>
<td>1 Vial</td>
<td>Lyophilized</td>
<td>Store 2–8°C DO NOT FREEZE</td>
</tr>
<tr>
<td>X-BACS™ Buffer</td>
<td>1 x 100mL</td>
<td>DPBS, 2mM EDTA, [-] Ca²⁺ [-] Mg²⁺</td>
<td>Store 2–30°C</td>
</tr>
<tr>
<td>Syringe 10mL</td>
<td>1</td>
<td>Sterile</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>21 G x 1 ½” Needle</td>
<td>2</td>
<td>Sterile</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>Syringe cap</td>
<td>10</td>
<td>Sterile</td>
<td>Room Temperature</td>
</tr>
</tbody>
</table>

Product Capacity: ~3.6 x 10⁸ PBMCs or 50mL Whole Blood

Use

All components are single use only. Expiration dates are indicated on the component labeling.

For research use only. Not intended for therapeutic or diagnostic use.

Symbol Legend:

- Refer to instructions
- Catalog Number
- Keep Dry
- Storage Temperature
- Lot number
- Expiration Date
- Manufactured By
- Caution

Description

The X-MINI® CD3 Selection Kit is intended for the selection of human CD3⁺ cells from peripheral blood mononuclear cell fraction or whole blood. The following protocol details the selection of CD3⁺ cells using a biotinylated antibody conjugate.

The X-MINI® CD3 Selection Kit may also be used to select other cell populations with biotinylated antibodies which may require optimization from the user. Selected cells are immediately available for downstream applications such as flow cytometry and cell culture expansion.

Principle

The basic target cell selection is completed in four steps:

1. Targeting of CD3⁺ cells from a prepared peripheral mononuclear cell (PBMC) fraction or whole blood using a biotinylated CD3 antibody.
2. Binding of streptavidin-coated microbubbles to biotinylated-target cells using proprietary X-BACS™ technology.
4. Removal of microbubbles from target cells using positive pressure to retrieve the target cell fraction.

Required Buffer:

Supplement X-BACS™ Buffer with serum for a final buffer concentration of 1% BSA or HSA, 2mM EDTA in Ca²⁺ and Mg²⁺ free DPBS.

Note: Refrigerate X-BACS™ Buffer at 2–8°C after supplementing with serum.

Materials Needed (not supplied):

- X-MINI Pressor (ThermoGenesis Corp., Catalog: 80094)
- BSA (Sigma-Aldrich, Catalog: A3299)
- Cell Strainer (Optional)
- 10mL Syringe (BD Biosciences, Catalog: 309604)
- Syringe Caps (BD Biosciences, Catalog: 305819)
- 2mL Microcentrifuge Tubes
- Rotating Mixer
- Saline (0.9% NaCl)

Precautions:

- Follow standard laboratory procedures when working with biological material, sharps, and biohazardous waste.
- Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- Reagents contained in this kit contain bovine serum albumin obtained from a commercial supplier. Information regarding the origin of the animal source used are maintained by the supplier and made available to ThermoGenesis. The position statements provided by the supplier state the sources of bovine material origin are from the USA, Canada, and Australia.
- The safety and effectiveness of this device for in vivo indications for use has not been established.

2. Preparation of PBMC Sample and X-BACS™ Reagent

If using whole blood, proceed with preparation of X-BACS™ Reagent and CD3 Cell Selection.

Preparation of PBMC

1. Prepare a single cell suspension of PBMC from whole blood by traditional methods or by using the X-LAB® System.

   Recommended: Filter aggregated suspensions through a cell strainer for optimal results.

2. After preparation, resuspend cells in X-BACS™ buffer at a cell density of up to 3 x 10⁷ cells/mL.

Preparation of X-BACS™ Reagent

Note: Before use, allow 15 minutes for X-BACS™ Reagent to come to room temperature (20–25 °C).

1. Insert the provided 21G 1 ½” needle through the septum of the X-BACS™ Reagent as a vent for pressure compensation.

2. Using the provided syringe and the second 21G 1 ½” needle, rapidly add 6 mL of saline to the vial.

3. Remove the syringe and vent needle from the vial.

   Note: Retain syringe for use in later steps.

4. Vigorously shake X-BACS Reagent for 10 seconds, and let the vial sit at room temperature for 5 minutes.

5. Mix the X-BACS™ Reagent again and insert the 21G 1 ½” needle through the septum for pressure compensation. Turn the vial upside down and insert a second 21G 1 ½” needle attached to a syringe to recover the desired volume of microbubbles.
3. CD3 Cell Selection

Cell labeling and selection volumes are for up to 3 x 10^6 total cells per PBMC sample or 1mL of whole blood. When working with cell numbers above 3 x 10^6 cells or more than 1mL of whole blood, scale up all reagent volumes and total volumes accordingly.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Instructions</th>
<th>PBMC</th>
<th>Whole Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mix sample preparation thoroughly and transfer cells to a 2mL microcentrifuge tube.</td>
<td>1mL</td>
<td>1mL</td>
</tr>
<tr>
<td>2</td>
<td>Add indicated volume CD3 antibody for each 1mL sample.</td>
<td>35μL</td>
<td>20μL</td>
</tr>
<tr>
<td>3</td>
<td>Mix at room temperature and incubate using a rotating mixer.</td>
<td>30 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td>4</td>
<td>After the incubation period, mix the X-BACS™ Reagent and add indicated volume to each sample tube.</td>
<td>500μL</td>
<td>120μL</td>
</tr>
<tr>
<td>5</td>
<td>Mix at room temperature and incubate using a rotating mixer to bind the microbubbles to the antibody-labeled cells.</td>
<td>20 minutes</td>
<td>10 minutes</td>
</tr>
<tr>
<td>6</td>
<td>Centrifuge cells for separation.</td>
<td>5 minutes at 400xg</td>
<td>Prior to spinning, add 500μL of supplemented X-BACS™ Buffer to tube. Spin 5 minutes at 500xg</td>
</tr>
<tr>
<td>7</td>
<td>During centrifugation, prepare syringe for degassing of the microbubbles. Remove the plunger from the 10 mL syringe and attach on a syringe cap. <strong>Note</strong>: Do not reinsert the plunger at this step.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Carefully pipette off white microbubble layer with a 200μL pipette and transfer to a pre-capped syringe.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Use X-BACS™ Buffer to wash any residual microbubbles adhering to the sides of the sample tube or pipette tip and add to the pre-capped syringe. <strong>Note</strong>: Do not disturb the cell pellet. The remaining pelleted cells are the unwanted (negative) cell fraction.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Increase the volume of the pre-capped 10 mL syringe containing the microbubble layer to a final volume of 3 mL with X-BACS™ Buffer. Replace the plunger.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Mix the sample thoroughly by inversion. Immediately proceed to the next step.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Degassing of the microbubble can be done using the X-MINI Pressor or manually. <strong>X-MINI Pressor</strong>: Follow the X-MINI Pressor product insert instructions. In brief: lift the handle and securely attach pre-capped syringe into the syringe holder. Place the cover firmly over the syringe and pull lever down to degas the microbubble-cell sample for 30 seconds. Lift the lever handle when done. <strong>Manual Degassing</strong>: Quickly and forcefully press the plunger down and hold for 30 seconds.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Repeat Steps 11 and 12 (for a total of 2 x 30 second degassing steps). The suspension will become less opaque.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>The selected cells are now ready for analysis and downstream applications. <strong>Note</strong>: For cell culture, wash cells and resuspend in complete media prior to culturing.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. X-MINI CD3 Selection Kit Data Example

Starting from human PBMCs, CD3^+ cells were selected using biotinylated CD3 antibody and X-BACS™ technology. The cells are fluorescently stained with CD3-PE. Cell debris and dead cells were excluded from analysis.

![Graph 1](image1.png)

![Graph 2](image2.png)

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