This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

**BACKGROUND**

B cells are a subset of lymphocytes that express immunoglobulin receptors which are tailored for specific antigens (1, 2). The B cell population is a vital component of the humoral immune response. B cells are involved in antigen presentation, cytokine homeostasis, and antibody production (3). The Human B Cell Expansion Kit contains the necessary components to induce human B cell proliferation. The kit contains sufficient reagents to expand a starting population of 10⁷ human B cells 3-5 fold.

**Figure 1: Improved B Cell Expansion using ExCellerate B Cell Media.** Human B cells were isolated from PBMCs and cultured for 10 days in either ExCellerate B Cell Media, or RPMI + 10% FBS. Both media conditions were supplemented with reagents included in the CellXVivo Human B Cell Expansion Kit. A) Fold expansion of B cells at 5 and 10 days in culture (n=3). B) Light microscopy of B cells at 20X magnification after 5 days of culture in ExCellerate B Cell Media.

**Figure 2: Detection of CD20 in Human B Cells.** Human B cells were isolated from PMBCs and cultured for 5 days in RPMI base media and using reagents included in the Human B Cell Expansion Kit. B cell expansion was measured with Resazurin (R&D Systems, Catalog # AR002).

**Figure 3: Human B Cell Expansion.** Human B cells were isolated from PMBCs and cultured for 5 days in RPMI base media and using reagents included in the Human B Cell Expansion Kit. B cell expansion was measured with Resazurin (R&D Systems, Catalog # AR002).

**Figure 4: Light Microscopy of B Cells.** (A) B cells were imaged at 20X magnification following isolation from PMBCs and (B) after 5 days of culture in RPMI base media and using reagents included in the Human B Cell Expansion Kit.
MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>PART #</th>
<th># VIALS</th>
<th>STORAGE OF OPENED/RECONSTITUTED MATERIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B Cell Expander 1</td>
<td>967572</td>
<td>1 vial</td>
<td>Store at 2-8 °C under sterile conditions for up to 30 days or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*</td>
</tr>
<tr>
<td>B Cell Expander 2</td>
<td>967573</td>
<td>1 vial</td>
<td>Store at 2-8 °C under sterile conditions for up to 3 months.*</td>
</tr>
<tr>
<td>B Cell Expander 3</td>
<td>967574</td>
<td>1 vial</td>
<td>Store at 2-8 °C under sterile conditions for up to 3 months.*</td>
</tr>
<tr>
<td>Reconstitution Buffer 1</td>
<td>967552</td>
<td>1 vial</td>
<td>Store at 2-8 °C under sterile conditions for up to 3 months.*</td>
</tr>
<tr>
<td>Reconstitution Buffer 2</td>
<td>967553</td>
<td>1 vial</td>
<td>Store at 2-8 °C under sterile conditions for up to 3 months.*</td>
</tr>
</tbody>
</table>

* Provided this is within the expiration date of the kit.

OTHER MATERIALS & SUPPLIES REQUIRED

- Ficoll-Hypaque™
- MagCellect™ Human B Cell Isolation Kit (R&D Systems, Catalog # MAGH103, or equivalent)
- ExCellerate B Cell Media (R&D Systems, Catalog # CCM031)
- RPMI 1640
- Fetal Bovine Serum (FBS)
- L-Glutamine/Penicillin/Streptomycin (optional)
- β-Mercaptoethanol (2-ME)
- Resazurin (R&D Systems, Catalog # AR002, or equivalent)
- Pipettes and pipette tips
- Tissue culture flasks and/or plates
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO₂ incubator
- Centrifuge

REAGENT PREPARATION

B Cell Expander 1 (500X) - Add 110 µL of Reconstitution Buffer 1 to B Cell Expander 1 to produce B Cell Expander 1 (500X).

B Cell Expander 2 (500X) - Add 110 µL of Reconstitution Buffer 1 to B Cell Expander 2 to produce B Cell Expander 2 (500X).

B Cell Expander 3 (500X) - Add 110 µL of Reconstitution Buffer 2 to B Cell Expander 3 to produce B Cell Expander 3 (500X).

PROTOCOL FOR B CELL EXPANSION

1. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.

2. Isolate human B cells from the PBMCs using the MagCellect Human B Cell Isolation Kit.

3. Suspend 2 x 10⁵ human B cells/mL in base media containing B Cell Expanders 1-3 as suggested in Table 1. **Note:** For base media we recommend using ExCellerate B Cell Media or RPMI 1640 supplemented with 10% FBS, 2 mM L-Glutamine, 50 µg/mL penicillin, 50 µg/mL Streptomycin, 50 µM β-mercaptoethanol.

4. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for 5 days.

5. Measure human B cell expansion using Resazurin.

Table 1: Suggested culture volumes for media and expanders.

<table>
<thead>
<tr>
<th>Size</th>
<th>Suggested Culture Volume</th>
<th>B Cell Expander 1-3 Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 cm² tissue culture flask</td>
<td>5 mL</td>
<td>10 µL each</td>
</tr>
<tr>
<td>75 cm² tissue culture flask</td>
<td>20 mL</td>
<td>40 µL each</td>
</tr>
<tr>
<td>6-well tissue culture plate</td>
<td>3 mL/well</td>
<td>6 µL each/well</td>
</tr>
<tr>
<td>24-well tissue culture plate</td>
<td>1 mL/well</td>
<td>2 µL each/well</td>
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</tbody>
</table>

REFERENCES