Differentiation of naïve CD4+ T cells into Th1 cells is confirmed by intracellular staining for IFN-γ (Figure 1) and secretion of IFN-γ (Figure 2). The corresponding tests for IL-4 (Th2 cell marker) and IL-17 (Th17 cell marker) are negative.

**REFERENCES**

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>Mouse Anti-Human CD3</td>
<td>967554</td>
<td>1 vial</td>
<td>May be stored 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>Human Th1 Reagent 1</td>
<td>967555</td>
<td>1 vial</td>
<td></td>
</tr>
<tr>
<td>Human Th1 Reagent 2</td>
<td>967556</td>
<td>1 vial</td>
<td></td>
</tr>
<tr>
<td>Reconstitution Buffer 1</td>
<td>967552</td>
<td>1 vial</td>
<td></td>
</tr>
<tr>
<td>Reconstitution Buffer 2</td>
<td>967553</td>
<td>1 vial</td>
<td></td>
</tr>
<tr>
<td>20X Wash Buffer</td>
<td>967557</td>
<td>3 vials</td>
<td></td>
</tr>
</tbody>
</table>

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

OTHER MATERIALS & SUPPLIES REQUIRED

- MagCellect™ Human Naïve CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGH115, or equivalent).
- Monesin
- PMA
- Ficoll-Hypaque™
- RPMI 1640
- L-Glutamine
- Ionomycin
- Penicillin
- Streptomycin
- Fetal Bovine Serum (FBS)
- β-Mercaptoethanol (2-ME)
- Tissue culture flasks and/or plates
- Microscope
- Hemocytometer
- 37 °C, 5% CO2 incubator
- Centrifuge
- Pipettes and pipette tips

REAGENT PREPARATION

Human Th1 Differentiation Media

1. Reconstitute Human Th1 Reagent 1 with 250 µL of Reconstitution Buffer 1, this is a 200X stock.
2. Reconstitute Human Th1 Reagent 2 with 250 µL of Reconstitution Buffer 1, this is a 200X stock.
3. Add 50 µL of Human Th1 Reagent 1 and 50 µL of Human Th1 Reagent 2 to 9.9 mL of cell culture media (RPMI, 2 mM L-Glutamine, 50 units/mL Penicillin, 50 µg/mL Streptomycin, 5% FBS, and 50 µM 2-ME).

Human CD3 Antibody

1. Reconstitute the Mouse Anti-Human CD3 antibody with 150 µL of Reconstitution Buffer 2, this is a 100X stock.
2. Add a 10 mL of 20X Wash Buffer to 190 mL of sterile deionized water to prepare 200 mL of 1X Wash Buffer.
3. Just before coating, dilute the 100X antibody stock 100-fold with 1X Wash Buffer.

PROTOCOL FOR Th1 DIFFERENTIATION

1. Coat a plate with Mouse Anti-Human CD3 antibody.
   a. For a 24-well plate, add 250 µL/well of diluted CD3 antibody. For a 96-well plate, add 50 µL/well of diluted CD3 antibody.
   b. Incubate at 2-8 °C overnight.
   c. Wash the plate with 1X Wash Buffer twice before use.
2. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
3. Isolate human naïve CD4+ T cells from human PBMCs using the MagCellect Human Naïve CD4+ T Cell Isolation Kit.
4. Suspend human naïve CD4+ T cells at 1-2 x 10^5 cells/mL in Human Th1 Differentiation Media.
5. Add the cells to a human CD3 antibody-coated plate. For a 24-well plate, add 1 mL/well. For a 96-well plate, add 0.2 mL/well.
6. Incubate the cells in a 37 °C, 5% CO2 humidified incubator for 5 days.
7. Collect media to analyze cytokine production profile.
8. Wash the cells once with RPMI, resuspend the cells in 1 mL of RPMI, 2 mM L-Glutamine, 50 units/mL penicillin, 50 µg/mL streptomycin, 10% FBS, 50 ng/mL PMA, and 1 µg/mL ionomycin. Incubate the cells in a 37 °C, 5% CO2, humidified incubator for 1 hour. Then add monesin at 3 µM and incubate for 3 hours.

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