**SUGGESTED REAGENTS FOR FLOW CYTOMETRY**

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<td>IC285A, and IC0041A</td>
<td>Human IFN-γ-APC MAb (Clone 25723), Mouse IgG, and Mouse IgG-APC Isotype Control (Clone 131303)</td>
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<td>IC204B, and IC002P</td>
<td>Human IL-4 Fluorescein MAb (Clone 1007), Mouse IgG, and Mouse IgG-Fluorescein Isotype Control (Clone 11711)</td>
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<td>FA13791S, and IC003F</td>
<td>Human CD4 Fluorescein MAb (Clone 11830), Mouse IgG, and Mouse IgG1 Fluorescein Isotype Control (Clone 20102)</td>
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<td>FC004</td>
<td>Flow Cytometry Fruation Buffer (1X)</td>
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<td>FC005</td>
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**REFERENCES**


**BACKGROUND**

T helper type 1 (Th1) cells are a lineage of CD4+ effector T cells that promote cell-mediated immune responses against intracellular viral and bacterial pathogens (1). Enhanced Th1 responses are associated with autoimmune diseases including rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and type-1 diabetes (2). Differentiation of CD4+ effector T cells into the Th1 lineage is promoted by cytokines such as IL-12 and IFN-γ (3). Th1 cells secrete IFN-γ, IL-10, and TNF-α. The Human Th1 Differentiation Kit contains all necessary components to differentiate human naïve CD4+ T cells into Th1 polarized cells.
MATERIALS PROVIDED & STORAGE CONDITIONS

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

COMPONENTS | PART #  | # VIALS | STORAGE OF OPENED/RECONSTITUTED MATERIAL
--- | --- | --- | ---
Mouse Anti-Human CD3 | 967554 | 1 vial | 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.*
Human Th1 Reagent 1 | 967555 | 1 vial | May be stored under sterile conditions for up to 3 months at 2-8 °C.*
Human Th1 Reagent 2 | 967556 | 1 vial | May be stored under sterile conditions for up to 3 months at 2-8 °C.*
Reconstitution Buffer 1 | 967552 | 1 vial | May be stored under sterile conditions for up to 3 months at 2-8 °C.*
Reconstitution Buffer 2 | 967553 | 1 vial | May be stored under sterile conditions for up to 3 months at 2-8 °C.*
20X Wash Buffer | 967557 | 3 vials | 20X Wash Buffer

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

OTHER MATERIALS & SUPPLIES REQUIRED

• Ficoll-Hypaque™
• MagCellect™ Human Naive CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGH115, or equivalent).
• RPMI 1640
• Fetal Bovine Serum (FBS)
• L-Glutamine/Penicillin/Streptomycin (optional)
• β-Mercaptoethanol (2-ME)
• Sterile deionized water
• Cell Activation Cocktail 500X (Tocris, Catalog #5476)
• Tissue culture flasks and/or plates
• Pipettes and pipette tips
• Inverted microscope
• Hemocytometer
• 37 °C, 5% CO₂ incubator
• Centrifuge

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 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 10⁵ 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 humified 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 and 1X Cell Activation Cocktail. Incubate the cells in a 37 °C, 5% CO₂ humified incubator for 4 hours.

PROTOCOL OUTLINE

Coat wells of a plate with Mouse Anti-Human CD3 Antibody.

Isolate PBMCs from human blood.

Isolate human naïve CD4+ T cells from PBMCs (e.g., using magnetic cell selection).

Perform a cell count.

Suspend 1-2 x 10⁵ naïve CD4+ T cells/mL in differentiation media supplemented with Human Th1 Reagents 1 and 2.

Culture the cells on plates pre-coated with CD3 antibody.

Verify Th1 cell differentiation by analyzing cytokine expression via flow cytometry.

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