Flow Cytometry Fixation & Permeabilization Buffer

Cell Activation Cocktail 500X (Tocris®)

SUGGESTED REAGENTS FOR FLOW CYTOMETRY

Table below.

Corresponding catalog numbers used in this figure are shown in the

Controls. All R&D Systems® and Novus Biologicals® antibodies and

Differentiated Th1 cells were stimulated with Cell Activation Cocktail

In this kit. After 6 days of differentiation, naïve CD4

Cells Secrete High Levels

IFN-γ IL-17 IL-4

Figure 2: Th1-differentiated Mouse CD4+ Cells Secrete High Levels of IFN-γ. Mouse naïve CD4+ T cells were differentiated for 6 days using the reagents included in this kit. On day 6 of differentiation cells were harvested and re-stimulated with Anti-Mouse CD3 and Anti-Mouse CD28 overnight. The cell culture supernatant was collected and cytokine secretion was determined using the Mouse IFN-γ Quantikine® ELISA Kit, the Mouse IL-4 Quantikine® ELISA Kit, and the Mouse IL-17 Quantikine® ELISA Kit. All relevant Quantikine® and DuoSet® ELISA kits and corresponding R&D Systems® catalog numbers are listed in the table below.

SUGGESTED REAGENTS FOR ELISA

<table>
<thead>
<tr>
<th>CATALOG #</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>M7700 or DY421</td>
<td>Mouse IL-17 Quantikine® ELISA Kit, or Mouse IL-17 DuoSet® ELISA</td>
</tr>
<tr>
<td>M8800 or DY485</td>
<td>Mouse IFN-γ Quantikine® ELISA Kit, or Mouse IFN-γ DuoSet® ELISA</td>
</tr>
<tr>
<td>M4000B or DY404</td>
<td>Mouse IL-4 Quantikine® ELISA Kit, or Mouse IL-4 DuoSet® ELISA</td>
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</tbody>
</table>

REFERENCES


Mouse Th1 Cell Differentiation Kit

Catalog Number: CDK018

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 CellXVivo™ Mouse Th1 Cell Differentiation Kit contains optimized reagents for Th1 differentiation from naive CD4+ cells. The quantity of components in this kit is sufficient to differentiate approximately 8 x 10⁶ naïve CD4+ T cells, and generate 8 x 10⁶ CD4+ cells of which ≥ 70% are IFN-γ Th1 polarized cells.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.
PROTOCOL FOR Th1 DIFFERENTIATION

The quantity of components in this kit is sufficient to differentiate approximately 8 x 10^6 naïve CD4+ T cells, and generate 8 x 10^7 CD4+ cells of which 70-90% are IFN-γ Th1 polarized cells.

**Note:** If starting with fewer cells, adjust starting volumes/number of wells accordingly.

1. Coat the desired tissue culture plate with Hamster Anti-Mouse CD3 (1X).
   a. Add Hamster Anti-Mouse CD3 (1X) to plate using the suggested coating volumes below.
   b. Incubate overnight at 2-8 °C or 2-3 hours at 37 °C.
   c. Wash plate or flask twice with Wash Buffer (1X) just prior to adding cells.

2. Prepare a single cell suspension of mouse splenocytes and isolate mouse naïve CD4+ T cells according to the product insert for the MagCellect™ Mouse Naïve CD4+ T Cell Isolation Kit. Perform a cell count.
   **Note:** 1 mouse spleen will provide roughly enough naïve CD4+ T cells for 1 well of a 24-well plate. The quantity of spleens needed may vary based on mouse strain, age, and/or health.

3. Suspend mouse naïve CD4+ T cells at 1x10^6 cells/mL in Mouse Th1 Differentiation Media.
4. Add the cells to a Hamster Anti-Mouse CD3 antibody-coated plate using the suggested volumes below.

5. Centrifuge the plate at 300 x g for 1 minute and incubate the cells in a 37 °C, 5% CO₂ incubator for 3 days.
6. On day 3 of differentiation, harvest cells and dilute them 1:10 by adding fresh Mouse Th1 Differentiation Media in an appropriate sized conical tube.
7. Transfer diluted cells to a new, uncoated plate or flask using the volumes indicated in the table below. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for an additional 3 days.

**PROTOCOL FOR Th1 DIFFERENTIATION CONTINUED**

8. On day 6 of differentiation, the differentiated mouse Th1 cells are ready to be used for downstream applications.
9. To verify Th1 cell differentiation via flow cytometry, collect the cells and wash with X-VIVO™ 15 Medium once, resuspend the cells in 1 mL X-VIVO™ 15 Medium and Cell Activation Cocktail (1X). Addition of Penicillin (100 units/mL) and Streptomycin (100 µg/mL) is recommended. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for 4-5 hours. Analyze cytokine expression via flow cytometry.

**PROTOCOL OUTLINE**

1. **Coat** the desired tissue culture plate with Hamster Anti-Mouse CD3, Th1 antibody.
2. **Isolate** mouse splenocytes.
3. **Perform** a cell count.
4. **Suspend** 1 x 10^6 naïve CD4+ T cells/mL in Mouse Th1 Differentiation Media.
5. **Harvest** cells on day 3.
6. **Verify** Th1 cell differentiation by analyzing cytokine expression via flow cytometry or ELISA (optional).

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