ImmunoCult™ Mouse Th1 Differentiation Supplement

Supplement for the differentiation of mouse naïve CD4+ T cells into Th1 cells

Catalog # 10953 1 mL

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Product Description

ImmunoCult™ Mouse Th1 Differentiation Supplement contains a combination of recombinant mouse cytokines and a blocking monoclonal antibody formulated to promote the differentiation of mouse naïve CD4+ T cells into Th1 cells.

This supplement is intended for use with RPMI 1640 Medium (Catalog #36750) containing fetal bovine serum and other additives, as well as anti-mouse CD3 and anti-mouse CD28 monoclonal antibodies as activating agents.

- Optimized for the induction of Th1 cells from naïve CD4+ T cells isolated from the spleen of a C57BL/6 mouse
- Supplied as a 100X concentrate; after thawing and mixing, the tube contents can be added directly to medium

Properties

Storage: Store at -20°C.

Shelf Life: Stable for 18 months from date of manufacture (MFG) on label.

Contains:
- Recombinant mouse interleukin 2 (IL-2)
- Recombinant mouse interleukin 12 (IL-12)
- Rat anti-mouse interleukin 4 (anti-IL-4)

Handling / Directions For Use

Please read the entire protocol before proceeding. Use sterile techniques when performing the following protocols.

A. COATING CULTUREWARE WITH ANTI-MOUSE CD3 ANTIBODY

Coat a flat-bottom tissue culture-treated plate (e.g. Catalog #38015) with anti-mouse CD3 antibody (e.g. Anti-Mouse CD3e Antibody, Clone 145-2C11, Catalog #60015) at a density of 312.5 ng/cm^2. Cover plate with plastic wrap and store at 2 - 8°C overnight.

B. PREPARATION OF Th1 DIFFERENTIATION MEDIUM

1. Thaw ImmunoCult™ Mouse Th1 Differentiation Supplement on ice until just thawed. Mix thoroughly.
   NOTE: If necessary, centrifuge vial for 30 seconds to recover liquid from cap.
   NOTE: If not used immediately, store at 2 - 8°C for up to 1 month. Do not re-freeze.
2. Add the following components to RPMI 1640 Medium (Catalog #36750) and mix thoroughly:
   - 5 - 10% fetal bovine serum
   - 2 mM L-Glutamine (Catalog #07100)
   - 10 mM HEPES Buffer Solution (Catalog #07200)
   - 1 mM sodium pyruvate
   - 100 µM MEM Non-Essential Amino Acid Solution (Catalog #07600)
   - 50 µM ß-mercaptoethanol
3. Add ImmunoCult™ Mouse Th1 Differentiation Supplement at a 1 in 100 dilution. Mix thoroughly.
   NOTE: If not used immediately, store at 2 - 8°C for up to 1 month.

C. DIFFERENTIATION TO Th1 CELLS

1. DAY 0: Remove and discard antibody from coated wells (prepared in section A). Wash coated wells twice with sterile phosphate-buffered saline.
2. Add anti-mouse CD28 antibody (recommended clone 37.51) to Th1 Differentiation Medium (prepared in section B) to a final concentration of 0.5 µg/mL. Mix thoroughly.

3. Isolate naïve CD4+ T cells using EasySep™ Mouse Naïve CD4+ T Cell Isolation Kit (Catalog #19765). Dilute cells to 5 x 10^5 cells/mL in Th1 Differentiation Medium + anti-mouse CD28 antibody (prepared in step 2).
   NOTE: ImmunoCult™ Mouse Th1 Differentiation Supplement is optimized for the polarization of naïve CD4+ T cells isolated from the spleen of C57BL/6 mice.
4. Add cell suspension to coated wells at a density of 3.12 x 10^5 cells/cm^2. Incubate at 37°C and 5% CO_2 for 3 days.
5. DAY 3: Add cells to fresh tissue culture-treated wells at a split ratio of 1:2 to 1:4. Add fresh Th1 Differentiation Medium (without anti-mouse CD28 antibody). Incubate at 37°C and 5% CO_2 for 2 days.
   NOTE: The cells do not need to be re-stimulated with anti-mouse CD3 and anti-mouse CD28 antibodies.
6. DAY 5: Th1 cells are ready to be assayed in the desired application.

Notes and Tips

For assessment of Th1 cells by flow cytometry the following fluorochrome-conjugated antibody clones can be used:

- Anti-mouse CD4 antibody, clone GK1.5
- Anti-mouse T-bet antibody, clone 4B10
- Anti-mouse IFN-gamma, clone XMG1.2