ImmunoCult™ Mouse Treg Differentiation Supplement

Supplement for the differentiation of mouse naïve CD4+ T cells into regulatory T cells (Tregs)

Catalog # 10957 1 mL

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Product Description

ImmunoCult™ Mouse Treg Differentiation Supplement contains a combination of recombinant mouse and human cytokines and a small molecule optimized to promote the differentiation of mouse naïve CD4+ T cells into regulatory T cells (Tregs).

This supplement is intended for use with RPMI 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 Tregs 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 12 months from date of manufacture (MFG) on label.

Contains:
- Recombinant mouse interleukin 2 (IL-2)
- Recombinant human transforming growth factor beta (TGF-β)
- All-trans retinoic acid (ATRA)

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 Treg DIFFERENTIATION MEDIUM

1. Thaw ImmunoCult™ Mouse Treg 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 (Catalog #07000)
   - 100 µM MEM Non-Essential Amino Acid Solution (Catalog #07600)
   - 50 µM ß-mercaptoethanol
3. Add ImmunoCult™ Mouse Treg 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 Tregs

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 Treg 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 Treg Differentiation Medium + anti-mouse CD28 antibody (prepared in step 2).
   NOTE: ImmunoCult™ Mouse Treg 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 6 days.
5. DAY 6: Tregs are ready to be assayed in the desired application.

Notes and Tips

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

- Anti-mouse CD4 antibody, clone GK1.5
- Anti-Mouse CD25 Antibody, Clone PC61.5 (Catalog #60009)
- Anti-mouse FoxP3 antibody, clone FJK-16s