DATA EXAMPLES

Differentiation of naïve CD4$^+$ T cells into Treg cells is confirmed by intracellular staining for FoxP3 (Figure 1).

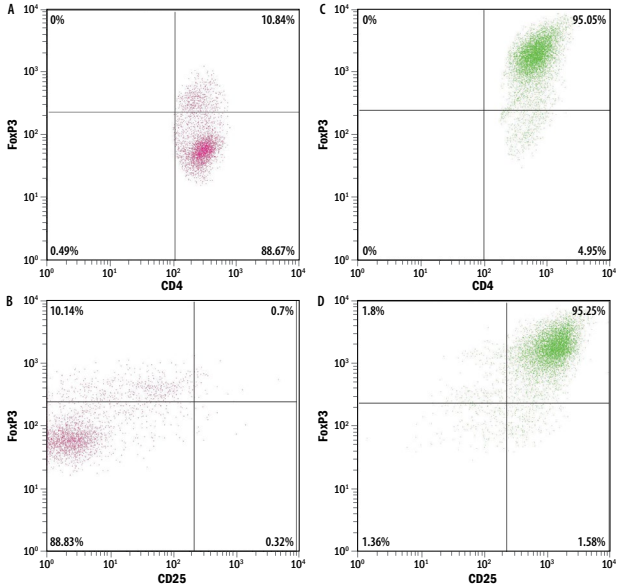

Figure 1: Intracellular Staining of Differentiated Treg Cells. Flow cytometry data showing mouse naïve CD4$^+$ T cells without (A, B) and with (C, D) a 5 day differentiation using reagents included in the Mouse Treg Cell Differentiation Kit. On day 5 of differentiation, the cells were fixed, permeabilized, and stained using the FlowX™ Mouse Regulatory T Cell Kit (R&D Systems, Catalog # FMC022). Quadrants were set based on isotope-stained samples.

REFERENCES

1. Feuerer, M. *et al.* (2009) Nat. Immunol. **10**:689.
2. Liston, A. and D.H. Gray (2014) Nat. Rev. Immunol. **14**:154.
3. Campbell, D.J. and M.A. Koch (2011) Nat. Rev. Immunol. **11**:119.

NOTES

CellXVivo™

Mouse Treg Cell Differentiation Kit

Catalog Number: CDK007

BACKGROUND

CD4$^+$ T cells differentiate into T helper cells under the influence of various cytokines and cellular interactions that induce expression of specific transcription factors. Naïve CD4$^+$ T cells can be induced to Forkhead Box P3 (FoxP3)$^+$ regulatory T (Treg) cells by activation in the presence of IL-2 and TGF-β *in vitro* (1). Treg cells are a suppressive subset of CD4$^+$ T cells that function to antagonize immune responses. Treg cells have the capacity to prevent potentially damaging autoimmune and protective immune responses, so the number of Treg cells is a crucial determinant of the regulatory burden on the immune system (2). Treg cells prevent autoimmune disease, maintain immune homeostasis, and modulate immune responses during infection (3). The Mouse Treg Cell Differentiation Kit contains specially formulated reagents and growth factors to differentiate mouse naïve CD4$^+$ T cells into FoxP3$^+$CD25$^+$ Treg cells.

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

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at -20 °C in a manual defrost freezer.
Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Hamster Anti-Mouse CD3	967818	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.*
Mouse Treg Reagent 1	967823	1 vial	
Mouse Treg Reagent 2	967824	1 vial	
Mouse Treg Reagent 3	967825	1 vial	May be stored at -20 °C to -70 °C under sterile conditions in a manual defrost freezer for up to 3 months.*
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	
20X Wash Buffer	967557	3 vials	

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

OTHER MATERIALS & SUPPLIES REQUIRED

- MagCellect™ Mouse Naïve CD4⁺ T Cell Isolation Kit (R&D Systems, Catalog # MAGM205, or equivalent)
- RPMI 1640
- Fetal Bovine Serum (FBS)
- β-Mercaptoethanol (2-ME)
- L-Glutamine–Penicillin–Streptomycin solution
- Tissue culture plates
- Sterile deionized water
- Microscope
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Centrifuge

REAGENT PREPARATION

Mouse Treg Differentiation Media

1. Reconstitute Mouse Treg Reagent 1 and Mouse Treg Reagent 2 each with 125 µL of Reconstitution Buffer 1; these are 400X stocks.
2. Add 62.5 µL each of Mouse Treg Reagents 1 and 2 and 25 µL of Mouse Treg Reagent 3 (provided as 1000X liquid stock) to 24.85 mL of cell culture media (RPMI, 2 mM L-Glutamine, 100 units/mL Penicillin, 100 µg/mL Streptomycin, 10% FBS, and 50 µM 2-ME).

Hamster CD3 Antibody

1. Reconstitute the Hamster Anti-Mouse CD3 antibody with 125 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. Approximately 6 mL/plate is required.

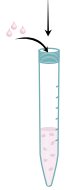
PROTOCOL FOR Treg DIFFERENTIATION

1. Coat a plate with Hamster Anti-Mouse CD3 antibody.
   a. For a 24-well plate, add 250 µL/well of diluted CD3 antibody. For a 6-well plate, add 1 mL/well of diluted CD3 antibody.
   b. Incubate at 2-8 °C overnight.
   c. Wash the plate with 1X Wash Buffer once before use.
2. Prepare a single cell suspension of mouse splenocytes using a traditional method.
3. Isolate mouse naïve CD4⁺ T cells from splenocytes using the MagCellect Mouse Naïve CD4⁺ T Cell Isolation Kit
4. Suspend mouse naïve CD4⁺ T cells at 0.5 -1 x 10^6/mL in Mouse Treg Differentiation Media.
5. Add the cells to a CD3 antibody-coated plate. For a 24-well plate, add 1 mL/well. For a 6-well plate, add 4 mL/well.
6. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator for 5 days.
7. After 5 days of differentiation, the differentiated Treg cells are ready for downstream applications.
8. **Optional:** To verify Treg cell differentiation via flow cytometry, collect the cells and wash with PBS once. Fix, permeabilize, and stain the cells using the FlowX™ Mouse Regulatory T Cell Kit following the instruction in the kit. Analyze FoxP3 and CD25 expression via flow cytometry as shown in the Data Examples.

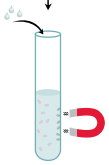
PROTOCOL OUTLINE

Coat wells of a 24-well plate with Hamster Anti-Mouse CD3 Antibody.

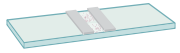
Prepare a single cell suspension of mouse splenocytes.



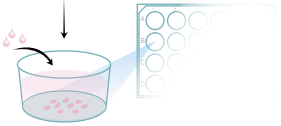
Isolate mouse naïve CD4⁺ T cells from splenocytes (e.g., using magnetic cell selection).



Perform a cell count.



Suspend 0.5-1.0 x 10^6 naïve CD4⁺ T cells/mL in Mouse Treg Differentiation Media. **Culture** the cells on plates pre-coated with CD3 antibody for 5 days.



Verify Treg cell differentiation by analyzing marker expression via flow cytometry **(optional)**.