# Cloudz™ Human Treg Expansion Kit

Catalog Number CLD006

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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## DESCRIPTION

The Cloudz Human Treg Expansion Kit is designed for the robust expansion of human regulatory T (Treg) cells from $CD4^+$ T cells. The kit contains dissolvable microspheres that are functionalized with anti-CD3 and anti-CD28 antibodies (Cloudz Treg CD3/CD28). The kit also contains Cloudz 6X Release Buffer which enables easy cell harvesting by quickly dissolving Cloudz Treg CD3/CD28. Each kit contains enough materials to activate and expand Tregs from $1 \times 10^8$ $CD4^+$ T cells, with greater than 500-fold expansion in 9 days.

## 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 into Forkhead Box P3 $(FoxP3)^+$ 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). Continued *in vitro* research on methods of Treg cell expansion and mechanisms of immunoregulation are necessary to elucidate their application in both preclinical and therapeutic settings.

## LIMITATIONS

- This reagent should not be used beyond the expiration date indicated on the label.
- Do not use if package is damaged. Use undamaged and sealed bottles only.
- Results may vary due to variations among cells derived from different donors.

## PRECAUTIONS

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

## MATERIALS PROVIDED & STORAGE CONDITIONS

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

| PART | PART # | AMOUNT PROVIDED | STORAGE OF OPENED/RECONSTITUTED MATERIAL |
|---|---|---|---|
| **Cloudz Treg CD3/CD28** | SPC-4265 | 16.5 mL | Store at 2-8 °C.* |
| **Cloudz 6X Release Buffer** | SPC-4262 | 100 mL | |

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

## OTHER MATERIALS REQUIRED

- ExCellerate™ Human T Cell Expansion Media, Xeno-free (R&D Systems, Catalog # CCM030)
- Fetal Bovine Serum (R&D Systems, Catalog # S11150)
- Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL or 202-GMP)
- MagCellect™ Human $CD4^+$ T Cell Isolation Kit (R&D Systems, Catalog # MAGH102)
- 24-well tissue culture plates
- T-25 and T-75 flasks
- Sterile 1X PBS
- Microscope
- Cell counter
- 37 °C, 5% $CO_2$ incubator
- Centrifuge
- Pipettes and pipette tips
- FlowX™ Human Regulatory T Cell Multi-Color Flow Kit (R&D Systems, Catalog # FMC021)

## REAGENT PREPARATION

**Complete Treg Cell Expansion Media** - Prepare Complete Treg Cell Expansion Media by supplementing ExCellerate Human T Cell Expansion Media with 10% FBS and Recombinant Human IL-2 (20 ng/mL).

**Note:** *FBS and cytokine-supplemented ExCellerate Human T Cell Expansion Media will be stable for 2 weeks when stored at 2-8 °C.*

**Cloudz 1X Release Buffer** - Immediately before use, dilute Cloudz 6X Release Buffer 1:6 using sterile 1X PBS to produce Cloudz 1X Release Buffer. (*i.e.,* add 10 mL of Cloudz 6X Release Buffer to 50 mL of Sterile 1X PBS to generate Cloudz 1X Release Buffer).

## PROTOCOL FOR HUMAN TREG CELL EXPANSION

The following protocol describes the expansion of human Treg cells from $CD4^+$ T cells in 24-well tissue culture plates using ExCellerate Human T Cell Expansion Media and Recombinant Human IL-2. This kit contains enough materials to activate and expand Treg cells from 1 x $10^8$ $CD4^+$ T cells, with greater than 500-fold expansion of $FoxP3^+$ cells in 9 days. Expansion rates may vary by donor.

**Note:** *Cloudz Human Treg Expansion Kit is compatible for use with other combinations of cytokines and base media. If alternate cytokines, cytokine concentrations, or media are desired, the expansion protocol should be optimized by application and/or experimental protocol.*

### SEEDING AND EXPANSION OF TREGS USING CLOUDZ TREG CD3/CD28

1. Obtain a purified population of human $CD4^+$ T cells using cell selection methods, such as the MagCellect Human $CD4^+$ T Cell Isolation Kit. Resuspend the selected cells in Complete Treg Cell Expansion Media.
2. Perform a cell count.
3. Seed $CD4^+$ cells at a concentration of 5 x $10^5$ cells/well in a 24-well plate. Add Complete Treg Cell Expansion Media to each well for a final volume of 2 mL/well.
4. Mix Cloudz Treg CD3/CD28 vial by vortexing for 30 seconds.
5. Add 75 µL of Cloudz Treg CD3/CD28 to each well containing cells. Gently pipette up and down 5 times to mix.
6. Place plates in a humidified incubator (37 °C, 5% $CO_2$). Culture for 9 days.
7. Every 2 to 3 days, perform a cell count. Maintain cell density around 5 x $10^5$ cells/mL by supplementing the cell culture with additional Complete Treg Cell Expansion Media. Transfer to a larger cell culture vessel as needed (*i.e.* T-25 anhd T-75 flask.)

### COLLECT EXPANDED TREG CELLS

1. Transfer entire cell culture from one well into a 15 mL conical tube (or appropriate centrifuge tube) and centrifuge at 300 x g for 5 minutes at room temperature.
2. Remove the supernatant. Re-suspend the cell pellet in 5 mL of Cloudz 1X Release Buffer. Pipette up and down 10 times to dissolve Cloudz Treg CD3/CD28.
3. Centrifuge at 300 x g for 5 minutes at room temperature.
4. Remove the supernatant and re-suspend the cell pellet in appropriate media or buffer for downstream analysis or applications.

   **Optional:** To verify Treg cell differentiation via flow cytometry, wash collected cells once using 1X PBS. Process, stain, and analyze the cells using the FlowX Human Regulatory T Cell Multi-Color Flow Kit. Analyze marker expression via flow cytometry as shown in the Data Examples.

## DATA EXAMPLES

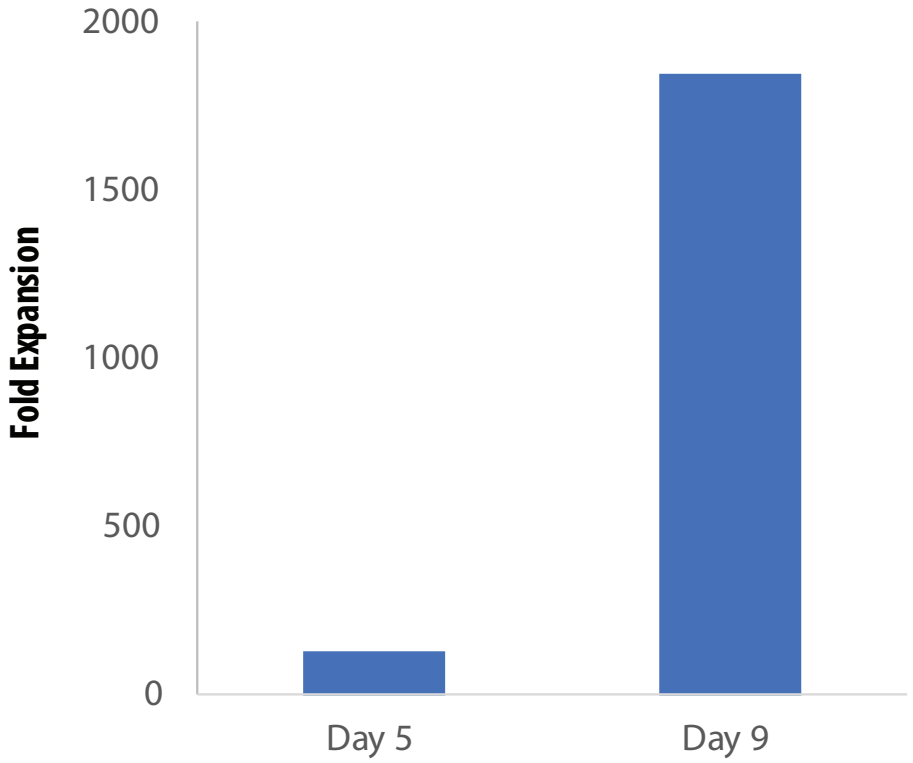


**Figure 1: Fold Expansion of Treg Cells using the Cloudz Human Treg Expansion Kit.**
Human $CD4^+$ T cells were cultured for 9 days following the protocol and using reagents included in the Cloudz Human Treg Expansion Kit. Expansion of FoxP3-positive Treg cells was evaluated using flow cytometry on Day 5 and Day 9 and fold expansion of more than 1500 was calculated.

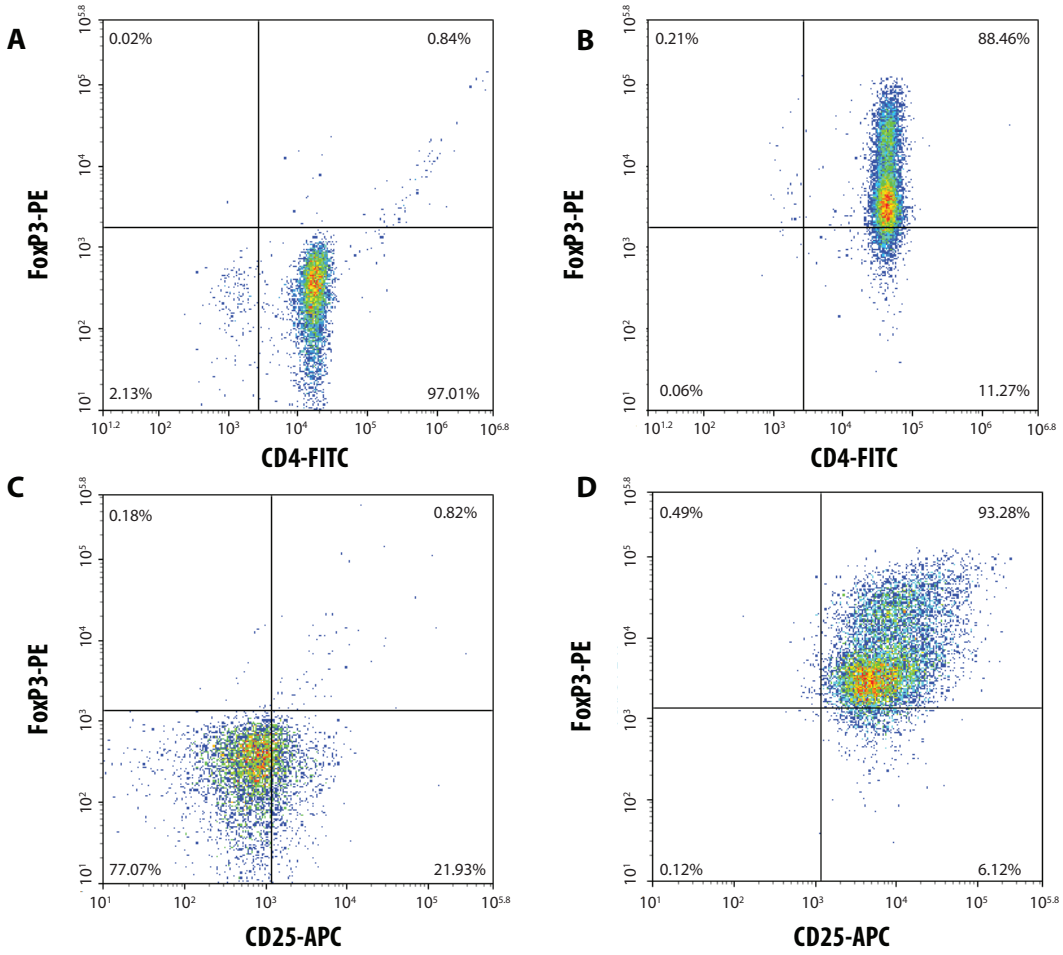


**Figure 2: Increased FoxP3-positive CD4$^+$ T Cells Following Treg Cell Expansion.** Flow cytometry data showing expression of the Treg cell marker, FoxP3, in CD4$^+$T cells on Day 0 **(A, C)** and Day 9 **(B, D)** following expansion using the Cloudz Human Treg Expansion Kit. Nine days after expansion, the cells were fixed, permeabilized, and stained with CD4-FITC, CD25-APC, and FoxP3-PE antibodies. Cells in panels **(A)** and **(B)** were stained with FoxP3 and CD4. Cells in **(C)** and **(D)** were stained with FoxP3 and CD25.

## 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.