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R&D SYSTEMS

# Regulatory T Cell (Treg)

*Multi-Color Flow Cytometry Panel*

**Catalog Number:** FMC-P-004

## PRODUCT DESCRIPTION

Regulatory T (Treg) cells are a critical subset of $CD4^+$ T cells that regulate the immune response and prevent autoimmunity. They are capable of inducing apoptosis, increasing cellular adenosine, and inhibiting DC maturation. This panel contains 6 conjugated antibodies that can be used for staining Tregs.

## INTENDED USE

This multicolor flow cytometry panel was validated on human peripheral blood mononuclear cells (PBMCs).

## MATERIALS PROVIDED & STORAGE

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

| SURFACE/INTRACELLULAR | MARKER | CLONE | FLUOROCHROME | SIZE | RECOMMENDED CONCENTRATION |
|---|---|---|---|---|---|
| Surface | CD3 | UCHT1 | Alexa Fluor® 405 | 100 tests | 5 µL/$10^6$ cells |
| | CD4 | 11830 | PerCP | 100 tests | 10 µL/$10^6$ cells |
| | CD25/IL-2R | 24212 | PE | 100 tests | 10 µL/$10^6$ cells |
| | CD127/IL-7R | 40131 | Alexa Fluor® 700 | 100 tests | 5 µL/$10^6$ cells |
| | GITR/TNFRSF18 | 110416 | FITC | 100 tests | 10 µL/$10^6$ cells |
| Intracellular | FoxP3 | 376209 | Alexa Fluor® 647 | 100 tests | 10 µL/$10^6$ cells |

***Note:*** *Recommended concentrations are given as reference point for antibody titration. Optimal concentrations should be determined by each laboratory for their experimental conditions.*

## PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## OTHER SUPPLIES REQUIRED

- PBS
- Flow Cytometry Staining Buffer (R&D Systems®, Catalog # FC001)
- Flow Cytometry Fixation Buffer (R&D Systems, Catalog # FC004)
- Flow Cytometry Permeabilization Buffer/Wash Buffer I (R&D Systems, Catalog # FC005)
- Fc-block (blocking IgG)
- (Optional; Isotype Control Antibodies)
- 5 mL Flow cytometry tubes

## PROTOCOL

### Surface Staining

1. Wash human PBMCs (1 x $10^6$ cells per sample) with 2 mL of Staining Buffer (1X) (R&D Systems® Catalog # FC001) or other BSA-containing buffer, by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes. Decant/aspirate supernatant.
2. Fc-block cells with blocking IgG (1 µg IgG/$10^6$ cells) for 10 minutes at room temperature.
3. Add previously titrated amount of each surface marker. Vortex tubes.
4. *(optional)* To separate tube, add 5 µL of each of the isotype control antibodies. Vortex tubes.
5. Incubate the mixtures for 30-45 minutes at room temperature **in the dark**.
6. Wash with 2 mL of Staining Buffer (1X), by spinning at 300 x g for 5 minutes at end of incubation. Decant/aspirate supernatant.



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**Global** bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
**Europe | Middle East | Africa** TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373

## PROTOCOL *continued*

### Intracellular Stain (with detergent permeabilization)

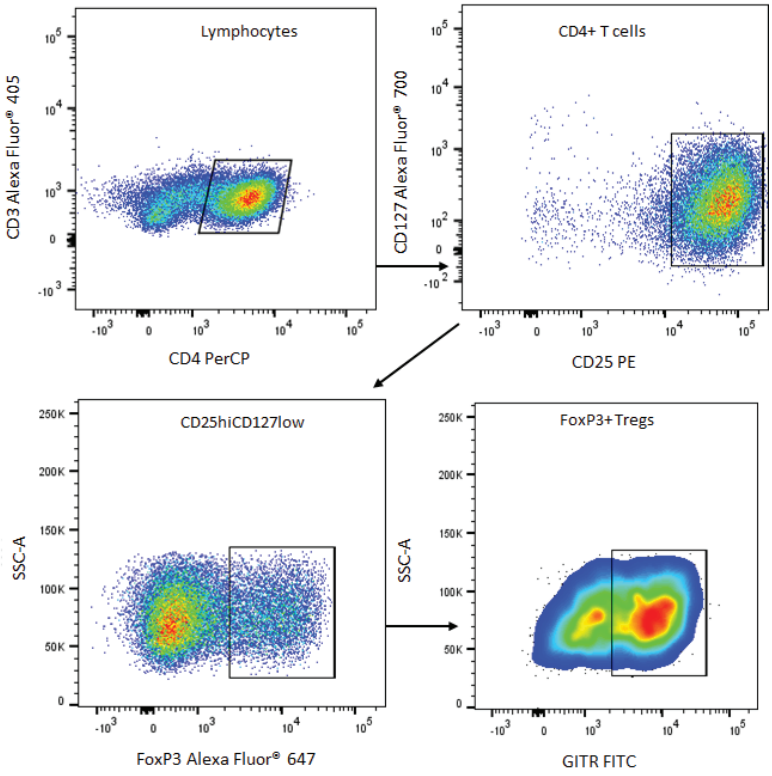
1. Add 0.5 mL of cold Flow Cytometry Fixation Buffer (R&D Systems® Catalog # FC004) and vortex. Incubate at room temperature for 10 minutes. Vortex cells intermittently to maintain a single cell suspension.
2. Centrifuge cells 350 - 500 x g for 5 minutes. Decant the Fixation Buffer.
3. Wash cells PBS (or HBSS) by adding 2 mL of PBS (or HBSS), centrifuge at 350-500 x g for 5 minutes, and decant buffer from pelleted cells. Repeat (2 total washes).
4. Resuspend the cell pellet in 0.1 - 0.2 mL of Flow Cytometry Permeabilization Buffer/Wash Buffer I (R&D Systems Catalog # FC005)

**Note:** *Saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of Permeabilization Buffer I during intracellular staining.*

**Note:** *Depending on the specific antibody and cell sample being used, the fixation and permeabilization steps can be performed simultaneously using Flow Cytometry Fixation/Permeabilization Buffer I (R&D Systems, Catalog # FC007).*

5. Add previously titrated amount of conjugated antibody to intracellular targets. Vortex tubes.
6. Incubate cells for 30 minutes at room temperature **in the dark**.
7. Wash cells 2 times with Flow Cytometry Permeabilization Buffer/Wash Buffer I (R&D Systems Catalog # FC005) as described in Step 3.
8. Resuspend the cells in 0.2 - 0.5 mL PBS (or HBSS) buffer and acquire on a Flow Cytometer.

## DATA EXAMPLES




**Multicolor flow cytometry panel to identify human T Regulatory Cells (Tregs).** Human T regulatory cells were expanded from PBMCs using Anti-Human CD3 Monoclonal Antibody (10 µg/mL, Catalog # MAB100) and Anti-human CD28 Monoclonal Antibody (5 µg/mL, Catalog # AF-342-PB) plus rhIL-2 (20 ng/mL, Catalog # 202-IL), rhTGF-β (10 ng/mL, Catalog # 7754-BH) for 2 days. Cells were stained with CD3 Alexa Fluor® 405, CD4 PerCP, CD25 PE, CD127 Alexa Fluor 700, FoxP3 Alexa Fluor 647, and GITR FITC. Tregs are $CD4^+$, $CD3^+$, CD25hi, CD127low, $Foxp3^+$. GITR is used as additional marker of human Tregs.