Foxp3 / Transcription Factor (Nuclear) Staining Protocol

Foxp3 / Transcription Factor Staining Buffer Kit Cat. No. TNB-0607-KIT

Kit Includes:

- Foxp3 / Transcription Factor Fix/Perm Concentrate (4X) Cat. No. TNB-1020-L050
- Foxp3 / Transcription Factor Fix/Perm Diluent (1X) Cat. No. TNB-1022-L160
- Flow Cytometry Perm Buffer (10X) Cat. No. TNB-1213-L150

Other Materials Required

- Flow Cytometry Staining Buffer (Stain Buffer) (1X PBS with 2% FBS, 0.09% Na-Azide)

Buffer and Solution Preparation

1. Prepare fresh Transcription Factor Fixation/Permeabilization working solution by diluting Transcription Factor Fixation/Permeabilization Concentrate (1 part) with Transcription Factor Fixation/Permeabilization Diluent (3 parts). You will need 1 mL of the Transcription Factor Fixation/Permeabilization working solution for each sample.
2. Prepare a 1X working solution of Flow Cytometry Perm Buffer by diluting the 10X concentrate with distilled water prior to use. You will need 3-5 mL of Flow Cytometry Perm Buffer working solution for each sample.

Experimental Procedure

1. Aliquot cell samples to tubes in a volume and at a cell concentration suitable for staining.
2. Stain cell surface antigen(s) with the recommended optimal concentration of fluorochrome labeled antibodies.

Tonbo Biosciences
4940 Carroll Canyon Road
San Diego, CA 92121-1735
(855) 848-6626 toll-free
(858) 888-7300 direct
(858) 888-7301 fax
info@tonbobio.com
www.tonbobio.com

3. Incubate for 20-30 minutes at 4°C or room temperature. Samples should be protected from light.
4. Wash cells with 1-2 mL Stain Buffer.
5. Centrifuge samples at 300-400 x *g* at room temperature for 5 minutes, discard the supernatant.
6. Vortex sample (<5 seconds) to completely dissociate the cell pellet.
7. Add 1 mL Transcription Factor Fixation/Permeabilization working solution to each tube and pulse vortex (< 5 seconds).
8. Incubate at 4°C or room temperature for 30-60 minutes in the dark.
9. Centrifuge samples at 300-400 x *g* at room temperature for 5 minutes, discard the supernatant.
10. Wash cells with 1-2 mL Flow Cytometry Perm Buffer working solution.
11. Centrifuge samples at 300-400 x *g* at room temperature for 5 minutes, discard the supernatant.
12. [Optional] Block with 2% normal mouse/rat serum by adding 2 µL directly to the cells. Incubate at room temperature for 15 minutes.
13. Without washing, add the recommended amount of fluorochrome-conjugated antibody for detection of intracellular antigen to cells and incubate in the dark at room temperature for at least 30 minutes.
14. Wash cells with 1-2 mL Flow Cytometry Perm Buffer working solution.
15. Centrifuge samples at 300-400 x *g* at room temperature for 5 minutes, discard the supernatant.
16. Wash cells with 1-2 mL Stain Buffer.
17. Centrifuge samples at 300-400 x *g* at room temperature for 5 minutes, discard the supernatant.
18. Resuspend stained cells in an appropriate volume of Stain Buffer and acquire data on a flow cytometer.

Tonbo Biosciences
4940 Carroll Canyon Road
San Diego, CA 92121-1735
(855) 848-6626 toll-free
(858) 888-7300 direct
(858) 888-7301 fax
info@tonbobio.com
www.tonbobio.com