Protocol

Foxp3 / Transcription Factor Staining Buffer Kit

Prepare working solutions:

* Fix/Perm Buffer:

· 1 mL needed for each sample of 1x10^6 cells; freshly prepared

· 1 part Transcription Factor Fixation/Permeabilization Concentrate (4X) (PF00011-A)

· 3 parts Transcription Factor Fixation/Permeabilization Diluent (PF00011-B)

* FC Perm Buffer:

· 4-5 mL needed for each sample

· Dilute Flow Cytometry Perm Buffer Concentrate (10X) (PF00011-C) to 1X with distilled water

* Additional Reagents Recommended:

· Flow Cytometry Staining Buffer (1X) (PF00018)

Proteintech Group, Inc.
(HQ in North America)

5500 Pearl Street, Ste 400
Rosemont, IL 60018, USA
P +1 (888) 478-4522 (toll free in USA)
F +1 (773) 598-4321
+1 (847) 928-2189
E proteintech@ptglab.com

Proteintech Europe

P +44 161 839 3007
F +44 161 241 3103
E europe@ptglab.com

Proteintech China

E proteintech-CN@ptglab.com

Proteintech Singapore

P +65 9785 0861
E singapore@ptglab.com

Protocol

Foxp3 / Transcription Factor Staining Buffer Kit

Protocol:

1. Prepare working solutions as described in the page above.

2. Stain any cell surface markers in the panel using the Proteintech Flow Cytometry Cell Surface Staining Protocol. (If panel does not include cell surface markers, skip this step.)

3. (After staining cell surface markers, wash and remove the supernatant.) For each tube of 1 x 10^6 cells, add 1 mL Fix/Perm Buffer. Gently pipette to fully suspend cells.

4. Incubate for 45 min at RT in the dark.

5. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant.

6. Add 2 mL FC Perm Buffer. Gently pipette to fully resuspend cells.

7. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant.

8. Resuspend cell pellet in 100 uL FC Perm Buffer and incubate for 10-15 min at RT in the dark.

9. Using the vendor recommended amount of antibody, for example 5 uL/test, (or pre-dilute flow cytometry antibodies with FC Perm Buffer to the optimal titration), incubate the cells with the antibodies for 30-45 min at RT in the dark to stain intracellular targets.

10. Wash cells by adding 2 mL FC Perm Buffer. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant.

11. Repeat step 10.

12. Resuspend cell pellet in 0.2 mL FC Perm Buffer.

13. Analyze cells with a flow cytometer.

Proteintech Group, Inc.
(HQ in North America)

5500 Pearl Street, Ste 400
Rosemont, IL 60018, USA
P +1 (888) 478-4522 (toll free in USA)
F +1 (773) 598-4321
+1 (847) 928-2189
E proteintech@ptglab.com

Proteintech Europe

P +44 161 839 3007
F +44 161 241 3103
E europe@ptglab.com

Proteintech China

E proteintech-CN@ptglab.com

Proteintech Singapore

P +65 9785 0861
E singapore@ptglab.com