Foxp3 / Transcription Factor Staining Buffer Set

Cat. No. NBP2-266911

Novus' FOXP3 staining kit is optimized for intracellular staining of cells in flow cytometric applications. It is designed and optimized to minimize non-specific staining while maximizing signal-to-noise ratio for clear and consistent data.

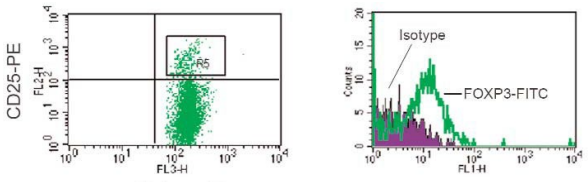
Kit Contents

Fixation/Permeabilization Concentrate: 30ml. Store at 2-8C. Avoid agitation. Use within 6 months of opening. This is a 4X stock solution that must be diluted prior to use with the Fixation/Permeabilization Diluent. Caution: This solution contains formaldehyde, which is toxic and a suspected carcinogen. Contact with eyes, skin and mucous membranes should be avoided. Wear proper protective clothing and gloves.

Fixation/Permeabilization Diluent: 100ml. Store at 4C. The diluent is intended to be used in combination with the Fixation/Permeabilization Concentrate.

Permeabilization Buffer (10X): 100ml. Store at 2-8C. Prior to use, this should be diluted 10-fold in distilled water. Note: The 10X Permeabilization Buffer has a natural tendency to precipitate, however, its function is not affected by this. To clarify, the solution can be filtered after dilution to a 1X working solution

NBP2-26668 FOXP3



Human PBMCs were stained with PE-conjugated anti-CD25 mAb and PerCPconjugated anti-CD4 mAb and subsequently the cells were permeabilized for intracellular staining with FITC-conjugated anti-FOXP3 mAb at a concentration of 0.5 µg per 1 x 10^6 cells. BD Biosciences mouse IgG1-FITC isotype control was used per manufacturers specification.

Preparation of working solutions

Fixation/Permeabilization buffer Prior to staining, dilute the Fixation/Permeabilization Concentrate (1 part) into the Fixation/Permeabilization Diluent (3 parts) to a desired volume of working solution. Working solution should not be stored for more than one day.

Permeabilization buffer It is supplied as a 10X solution and can be diluted with deionized water to its final 1X working concentration.

1. Determine the number of cells required for staining. For each test sample, it is recommended to use at least 1 x 106 cells. The following controls are suggested: a) unstained cells [no primary or secondary antibody staining], b) cells with an isotype control antibody, c) cells with a positive control antibody.
2. Harvest the cells and spin down to a pellet at 1000 RPM for 10 min. and decant supernatant.
3. Depending on the size of the pellet, resuspend in 2-3 ml of 1X PBS. An exact volume is not necessary at this step.
4. Count the cells with a hemocytometer. Remove 1 x 106 cells for each sample (including controls) to be tested to a clean conical centrifuge tube. Add 1 ml of 1X PBS to make the decanting easier.
5. Centrifuge cells at 1000 RPM for 10 min and decant supernatant.
6. Tap the conical tube gently to loosen the pellet.
7. Resuspend pellet with the appropriate volume of staining buffer or PBS (50 µl per 1 x 106 cells).
8. Aliquot 50 µl of cell suspension to labeled flow tubes.
9. Stain surface molecules CD4 and CD25 except for tube with unstained cells. Gently mix and incubate on ice for 30 min in the dark.
10. Wash with 2 ml cold Staining buffer (or cold PBS).
11. Centrifuge at 1000 RPM for 10 min. and decant supernatant.
12. Resuspend cell pellet with pulse vortex and add 1 ml of freshly prepared Fixation/Permeabilization working solution to each sample. Pulse vortex again. Incubate at 4°C for 30-60 min in the dark.
13. Wash once by adding 2 ml of 1X Permeabilization buffer.
14. Centrifuge at 1000 RPM for 10 min. and decant supernatant.
15. Optional: Block with 2% mouse serum by adding 2 µl to residual buffer after decanting (~100 µl) and incubate at 4°C for 15 min.
16. Without washing after blocking step, add fluorochrome conjugated anti-FOXP3 antibody or isoptype control in 1X Permeabilization buffer (~40 µl total) and incubate at 4°C for at least 30 min in the dark. Please perform further titration for optimal staining in your own assay system.
17. Wash cells with 2 ml of 1X Permeabilization buffer. Centrifuge and decant supernatant.
18. Repeat Step 18.
19. Resuspend in appropriate volume Staining buffer and analyze on flow cytometer following manufacturer's recommendations. Note: If not analyzing on the same day, samples can be stored overnight, in the dark, at 4°C. Due to the fixation and permeabilization procedure, the FSC/SSC distribution of the cell population will be different than live cells. Therefore the gate and voltages will need to be modified.