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Cat. No. NBP2-29493 Mouse FOXP3 (APC Conjugate) Staining Assay

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

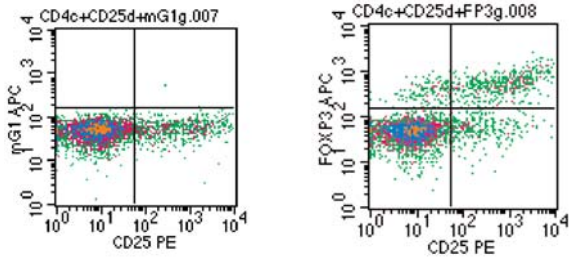
KC-500	Fixation/Permeabilization Concentrate, 4X	15 ml
KC-501	Fixation/Permeabilization Diluent, 1X	50 ml
KC-502	Permeabilization buffer, 10X	50 ml
KC-124	Staining buffer, 1X	3 X 60 ml
NBP2-26584	mCD4 FITC	0.5 ml
NBP2-27426	mCD25 PE	0.5 ml
NBP2-26671	FOXP3 APC (Clone 3G3)	0.25 ml
NBP2-24977	mouse IgG1 APC Isotype	0.25 ml
	Mouse Fc block	50 µl

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 the 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.

Mouse FOXP3



FOXP3 staining performed using Novus FOXP3 staining kit NBP2-29493. Mouse splenocytes were stained with anti-mCD4 FITC and anti-mCD25 PE, followed by fixation, permeabilization, and staining using anti-FOXP3 APC. CD4+ cells were gated and read for mCD25 PE against either APC isotype control (left) or FOXP3 APC (right).

1. Determine the number of cells required for staining. For each test sample, it is recommended to use at least 1 x 10^6 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 10^6 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 10^6 cells).
8. Aliquot 50 µl of cell suspension to labeled flow tubes.
9. Stain surface molecules CD4 and CD25 by adding 10 µl of each conjugate to your sample tubes, 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.
13. Incubate at 4°C for 30-60 min in the dark.
14. Wash once by adding 2 ml of 1X Permeabilization buffer.
15. Centrifuge at 1000 RPM for 10 min. and decant supernatant.
16. ***Optional:*** Block by adding 2 µl (1 µg) Fc block to residual buffer after decanting (~100 µl) and incubate at 4°C for 15 min.
17. Prepare an anti-FOXP3 and isotype control by adding 10 µl FOXP3 or 10 µl isotype control to 40 µl Permeabilization buffer. Without washing blocking step, add FOXP3 or isotype control to cells and incubate at 4°C for 30-40 min. in the dark (it is not necessary to wash after blocking step). Please perform further titration for optimal staining in your own assay system.
18. Wash cells with 2 ml of 1X Permeabilization buffer. Centrifuge and decant supernatant.
19. Repeat Step 18.
20. 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.*

Caution: *Fixation buffer contains paraformaldehyde which is toxic by inhalation, skin contact, or swallowing. Permeablization and staining buffers contain 0.05% sodium azide. 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 1X working solution. Use caution when handling. All the materials included in this kit should be treated as hazardous materials and be disposed of accordingly.*