

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 2-8°C. 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 4°C. The diluent is intended to be used in combination with the Fixation/Permeabilization Concentrate.

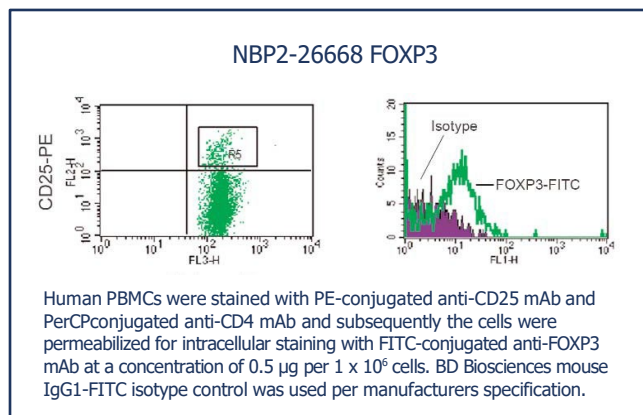
Permeabilization Buffer (10X): 100ml. Store at 2-8°C. Prior to use, this should be diluted 10-fold in distilled water. Note: The 10X Permeabilization Buffer has a natural tendency to precipitate, however, its function is not affected by this. To clarify, the solution can be filtered after dilution to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

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.

- Determine the number of cells required for staining. For each test sample, it is recommended to use at least 1×10^6 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.
- Harvest the cells and spin down to a pellet at 1000 RPM for 10 min. and decant supernatant.
- Depending on the size of the pellet, resuspend in 2-3 ml of 1X PBS. An exact volume is not necessary at this step.
- Count the cells with a hemocytometer. Remove 1×10^6 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.
- Centrifuge cells at 1000 RPM for 10 min and decant supernatant.
- Tap the conical tube gently to loosen the pellet.
- Resuspend pellet with the appropriate volume of staining buffer or PBS (50 μ l per 1×10^6 cells).
- Aliquot 50 μ l of cell suspension to labeled flow tubes.
- Stain surface molecules CD4 and CD25 except for tube with unstained cells. Gently mix and incubate on ice for 30 min in the dark.
- Wash with 2 ml cold Staining buffer (or cold PBS).
- Centrifuge at 1000 RPM for 10 min. and decant supernatant.
- 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.
- Wash once by adding 2 ml of 1X Permeabilization buffer.
- Centrifuge at 1000 RPM for 10 min. and decant supernatant.
- Optional: Block with 2% mouse serum by adding 2 μ l to residual buffer after decanting (~100 μ l) and incubate at 4°C for 15 min.
- Without washing after blocking step, add fluorochrome conjugated anti-FOXP3 antibody or isotype control in 1X Permeabilization buffer (~40 μ l 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.
- Wash cells with 2 ml of 1X Permeabilization buffer. Centrifuge and decant supernatant.
- Repeat Step 18.
- 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.