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# Intracellular Staining Flow Assay Kit

Catalog No. NBP2-26248

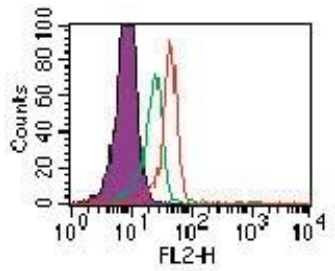
This kit is optimized for intracellular staining of cells in flow cytometric applications. It is designed and optimized to minimize non-specific staining while maximizing signal-to-noise ratio for clear and consistent data.

| Kit Contents (Store at 4°C) | |
|---|---|
| KC-136 Staining Buffer | 2 X 60 mL |
| KC-137 Fixation Buffer | 1 X 60 mL |
| KC-138 Permeabilization Buffer | 2 X 60 mL |

## Background Information

Fixation Buffer is used to "fix" the cells/cellular proteins prior to permeabilization.

Permeabilization Buffer permeabilizes the cell membrane, allowing the detecting antibodies access to intracellular proteins. It is supplied as a 10X solution and can be diluted with deionized water to its final 1X working concentration.




Intracellular flow cytometric analysis of TLR3 in human PBMCs using Novus anti-TLR3 monoclonal antibody (NBP2-24902) and intracellular staining flow kit. The shaded histogram represents cells without antibody; green represents an isotype control (Cat. No.: NBP2-24976); red represents anti-TLR3 antibody.

## Protocol

1. Determine the number of cells required for staining. For each test sample, a final concentration of 1 x $10^6$ cells in 50 µL of staining buffer will be needed. (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; 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 (50 µL per 1 x $10^6$ cells) of Fixation Buffer. Incubate at room temperature for 30 min
8. Centrifuge cells at 1000 RPM for 10 min and decant supernatant.
9. Resuspend pellet with the appropriate volume (50 µL per 1 x $10^6$ cells) of 1X Permeabilization Buffer.
10. Dispense 1x$10^6$ cells (50 µL) to the desired number of flow cytometer compatible test tubes and centrifuge cells at 1000 RPM for 10 min. Carefully aspirate the supernatant. During centrifugation, the primary antibodies to be used can be diluted to the required concentration in 50 µL of Permeabilization Buffer.
11. Resuspend each cell pellet with the appropriate primary antibody. Pipette up and down to thoroughly mix the antibody/cell suspension.
12. Incubate at room temperature for 30 min (protect from light if using a fluorescent-labeled primary antibody).
13. Centrifuge at 1000 RPM for 10 min and carefully aspirate supernatant.
14. Note: If using a fluorescent-labeled primary antibody, skip Steps 14-17.
15. Wash the cells by resuspending each cell pellet with 2 mL of Permeabilization buffer, centrifuge at 1000 RPM for 10 min, and decant supernatant. While centrifuging, dilute secondary antibody (FITC, PE or Biotin labeled) in 50 µL of Permeabilization Buffer per sample.
16. Resuspend cells in diluted secondary antibody.
17. Incubate at room temperature (protected from light) for 30 min.
18. Centrifuge at 1000 RPM for 10 min and carefully aspirate supernatant.
19. Wash cells twice with 2 mL of Permeabilization Buffer, centrifuging and decanting after each wash step.
20. After the final decanting, add 1 mL of Staining Buffer to each tube. Note: If not analyzing on the same day, samples can be stored overnight, in the dark, at 4°C.
21. Test samples on a flow cytometer following manufacturer recommendations.

*This product is guaranteed for six months from the date of purchase. Caution: Fixation buffer contains paraformaldehyde which is toxic by inhalation, skin contact, or swallowing. Permeabilization and staining buffers contain 0.05% sodium azide. All the materials included in this kit should be treated as hazardous materials and be disposed of accordingly.*