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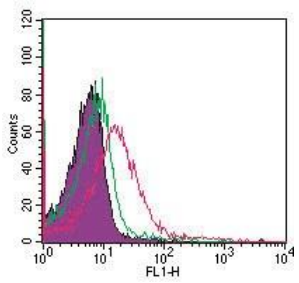
Cell Surface Staining Flow Assay Kit

Catalog No. NBP2-26247

Novus' Cell Surface staining flow kit is a convenient way to quickly and efficiently prepare samples for flow cytometric analysis.

Kit Contents (Store at 4 °C)

KC-136	Staining Buffer	3 x 60 mL
KC-125	Paraformaldehyde 10%	10 mL



Cell surface flow cytometric analysis of TLR4 in mouse splenocytes using Novus' anti-TLR4 monoclonal antibody (NBP2-24772) and cell surface staining kit. The shaded histogram represents cells alone; green represents isotype control; red represents anti-TLR4 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. Spin down cells to a pellet at 1000 RPM for 10 min and decant supernatant.
6. Tap the conical tube gently to loosen the pellet.
7. Resuspended pellet with an appropriate volume (50 µL per 1 x 10^6 cells) of Staining buffer. Aliquot 50 µL of cell suspension to individual flow cytometer compatible tubes, one aliquot for each sample to be tested.
8. To wash cells, add 1 mL of Staining buffer to each tube, spin down cells to a pellet at 1000 RPM for 10 min and decant supernatant. Prepare primary antibody solutions during centrifugation.
9. Dilute each antibody to be tested to the desired concentration in 50 µL of staining buffer. Resuspend each cell pellet with the appropriate primary antibody Pipette up and down to thoroughly mix the antibody/cell suspension.
10. Incubate on ice for 30 min (protect from light if using a fluorescent labeled primary antibody).
11. Centrifuge at 1000 RPM for 10 min and decant supernatant.
12. Note: If using a fluorescent-labeled primary antibody, skip Steps 12-14.
13. Wash the cells by resuspending each cell pellet with 2 mL of staining 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 Staining buffer per sample.
14. Resuspend cells with diluted secondary antibody.
15. Incubate on ice (protected from light) for 30 min. Centrifuge at 1000 RPM for 10 min and decant supernatant.
16. Wash cells twice in 2 mL of Staining Buffer, centrifuging and decanting after each wash step.
17. After the final decanting, add 1 mL of Staining Buffer to each tube. Note: If not analyzing on the same day, resuspend cells in 1% paraformaldehyde in Staining Buffer to "fix" cells, and store overnight at 4 °C. The Fixation Buffer can be removed, and the cells prepared for analysis by repeating step 15 and adding 1 mL of Staining buffer to each tube.
18. Test samples on a flow cytometer following manufacturer recommendations.

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