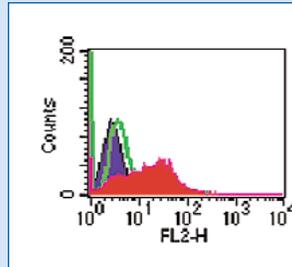


Cell Surface Staining Flow Protocol

Necessary Reagents

KC-124 Staining buffer, 1X	3 X 60 ml
KC-125 Paraformaldehyde 10%	1 X 10 ml



Flow cytometric analysis of cell surface TLR2 (IMG-416D) in ThP-1 cells using IMGENEX's CS-Flow kit (**Purple**: Unstained ThP-1 cells, **Green**: Isotype control, 2 μ g/10⁶ ThP-1 cells, **Red**: Anti-TLR2-PE, 2 μ g/10⁶ ThP-1 cells).

- Determine the number of cells required for staining. For each test sample, a final concentration of 1 x 10⁶ cells in 50 μ l 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.
- Harvest the cells and spin down to a pellet at 1000 RPM for 10 minutes; 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 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.
- Spin down cells to a pellet at 1000 RPM for 10 minutes and decant supernatant.
- Tap the conical tube gently to loosen the pellet.
- Resuspended pellet with an appropriate volume (50 μ l per 1 x 10⁶ cells) of Staining buffer. Aliquot 50 μ l of cell suspension to individual flow cytometer compatible tubes, one aliquot for each sample to be tested.
- To wash cells, add 1 ml of Staining buffer to each tube, spin down cells to a pellet at 1000 RPM for 10 minutes and decant supernatant. Prepare primary antibody solutions during centrifugation.
- Dilute each antibody to be tested to the desired concentration in 50 μ l of staining buffer. Resuspend each cell pellet with the appropriate primary antibody. Pipette up and down to thoroughly mix the antibody/ cell suspension.
- Incubate on ice for 30 minutes (protect from light if using a fluorescent labeled primary antibody).
- Centrifuge at 1000 RPM for 10 minutes and decant supernatant.

Note: If using a fluorescent-labeled primary antibody, skip Steps 12-14.

- Wash the cells by resuspending each cell pellet with 2 ml of staining buffer, centrifuge at 1000 RPM for 10 minutes, and decant supernatant. While centrifuging, dilute secondary antibody (FITC, PE or Biotin labeled) in 50 μ l of Staining buffer per sample.
- Resuspend cells with diluted secondary antibody.
- Incubate on ice (protected from light) for 30 minutes. Centrifuge at 1000 RPM for 10 minutes and decant supernatant.
- Wash cells twice in 2 ml of Staining buffer, centrifuging and decanting after each wash step.
- 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.
- Test samples on a flow cytometer following manufacturer recommendations.

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

>> For Intracellular Flow Kit please see Cat. No. 10084K