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17-1013: CellSurface FLOW Staining Kit

Application : FACS

Description

The Cell Surface FLOW Staining Kit allows the detection of antibodies on the cell surface proteins in flow cytometry analysis. This kit is specially formulated to reduce non-specific staining of fluorochrome-labelled antibodies and to increase fluorescence signaling. This buffer can be used for cell and antibody dilution steps, as well as wash steps for the cell surface staining and flow cytometric analysis. This buffer contains fetal bovine serum (FBS) to help reduce the non-specific binding of antibodies.

Product Info

Amount :	1 kit
Content :	Staining Buffer 1X (2 X 120mL) Fixation Buffer 10% (15mL)
Storage condition :	Store at 2-8°C

Application Note

Protocol:

1. Determine number of cells required for staining. Each sample contains 0.5 -1 x 10^6 cells in 50 µl of media or Staining Buffer. The following controls are needed for the experiment. Unstained cells (no antibodies were added), cells with isotype control and cells with secondary antibody (if secondary antibody was used).

2. Cetrifuge cells at 1000 RPM for 10 minutes and decant supernatant.

3. Resuspend pellet with appropriate volume of 1X Staining Buffer.

4. Aliquote 1x10^6 cells in 50 µl to the desired number of flow tubes. Dilute primary antibodies in 50 µl of 1X Staining Buffer. Add diluted antibodies into 50 µl of cells. Mix antibodies in cells suspension thoroughly.

5. Incubate in ice for 30 minutes. Wash cells in 2-3 ml of 1X Staining Buffer. Centrifuge 1000 RPM for 10 minutes. Decant supernatant carefully.

If primary antibody is fluorescent-labeled, resuspend pellet in 300-400 µl of Staining Buffer, analyze samples. Protected from light.

If primary antibody is not labeled, proceed with step 6.

6. Dilute secondary antibody (PE, FITC, APC or biotin labeled) in 50 µl Staining Buffer per sample.

7. Resuspend cells in diluted secondary antibody. Incubate in ice for 30 minutes. (protected from light).

8. Wash cells in 3-4 ml of staining buffer. Cetrifuge at 1000 RPM for 10 minutes. Decant supernatant.

9. After decanting, add 300-400 µl of Staining Buffer to each tube.

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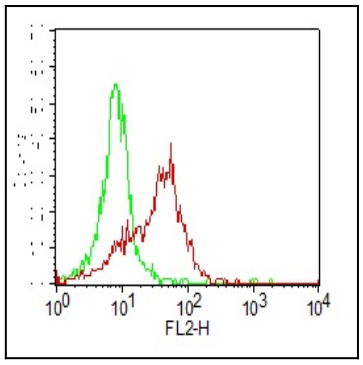
If not analyzing same day, resuspend cells in 1% Fixation Buffer (10% can be diluted to 1% using staining buffer). Samples can be stored over night in dark at 4°C.

Samples can be analyzed in flow cytometer according to the manufacturer protocol.



Cell Surface Staining of PBMC. 0.5 µg antibody was used. Green: Isotype control (mouse IgG1 PE). Red: human TLR2 PE (10-3026PE). Cell surface staining kit from ABEOMICS was used.