9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

17-1012: FLOW IntraCellular Staining Kit (IC FLOW)

Application : FACS

Description

The Intracellular FLOW Staining Kit allows detection antibodies to access the intracellular protein in Flowcytometry analysis. This kit is specially formulated to reduce non-specific staining of fluorochrome-labelled antibodies and to increase fluorescence signaling. First, live cells can be fixed with the Fixation buffer, which cross-links the protein. Second, the 1X Permeabilization Buffer creates holes in the membrane, allowing the antibodies enter into the cells effectively. Washing steps and antibody dilutions are done using 1X Permeabilization Buffer. The final cell resuspension prior to analysis is done using The Staining Buffer. **Permeabilization Buffer is supplied as a 10X solution. Prior to use, this should be diluted 10 fold in distilled water. (1ml of Permeabilization Buffer with 9 ml of distilled water). 10X Permeabilization buffer has natural tendency to precipitate, however, its function is not affected. 1X solution can be filtered.**

Product Info

Amount :	1 kit
Content :	Kit Components: Fixation Buffer (1X) - 60 ml, Permeabilization Buffer (10X)- 2 X 60 ml, Staining Buffer - (1X) - 60 ml
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 cells with appropriate volume of Fixation buffer. Incubate in ice for for 30 minutes.

4. Centrifuge cells 1000 RPM for 10 minutes and decant supernatant.

5. Resuspend pellet with appropriate volume of 1X permeabilization buffer.

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

7. Incubate in ice for 30 minutes. Wash cells in 2-3 ml of 1X permeabilization buffer. Centrifuge 1000 RPM for 10 minutes. Decant supernatant carefully.

If primary antibody is fluorescent-labeled, resuspent pellet in 300-400 µl of staining buffer, analyze samples. Protected from light.

If primary antibody is not labeled, proceed with step 8.

8. Dilute secondary antibody (PE, FITC, APC or biotin labeled) in 50 µl of permeabilization buffer per sample.

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

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

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

If not analyzing same day, samples can be stored over night in dark at 4 degrees C.

Samples can be analyzed in Flow Cytometer according to the manufacturer protocol.

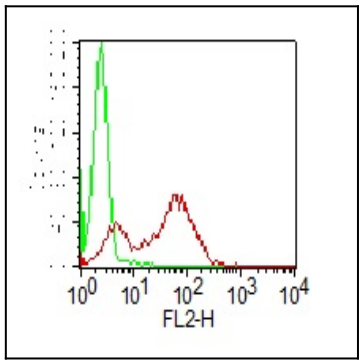

Figure-1: Intracellular Staining of PBMC. 0.5 µg of antibody was used. Red: Human TLR3 PE (10-3004PE). Green: Isotype control (mouse IgG1PE) ABEOMICS was used. Intracellular FLOW kit from ABEOMICS was used.

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