Flow Cytometry Phosphorylated Protein Staining Protocol

Reagents required :

Flow Cytometry Phosphorylated Protein Fixation/Permeabilization Kit (PF00026)

Deionized water

1X PBS

Flow cytometry antibodies

Experiment procedures:

1. Bring all buffers to working temperature prior to use (see kit components table).

2. Wash cells with PBS. Discard the supernatant.

3. Add 0.5 mL Fixation Buffer. Mix well and incubate at room temperature for 15 minutes.

4. Centrifuge at 350-500 x g for 5 minutes and aspirate (do not pour off) the supernatant.

5. Add 0.5 mL pre-cooled (-20°C) Permeabilization Buffer. Mix well by pipetting and incubate at room temperature for 5 minutes.

6. Centrifuge at 350-500 x g for 5 minutes and aspirate (do not pour off) the supernatant.

7. Dilute the 10X Staining Buffer to 1X with deionized water.

8. Add 1.5 mL 1X Staining Buffer. Mix well and centrifuge at 350-500 x g for 5 minutes and aspirate (do not pour off) the supernatant.

9. Repeat wash step 8.

10. Resuspend cells in 100 uL 1X Staining Buffer.

11. Add the recommended amount of primary antibody and incubate for 20-40 minutes at 4°C in the dark.

12. Wash cells twice as in step 8.

Note: If using fluorochrome-conjugated primary antibodies, skip to step 15.

13. Resuspend the cells with diluted fluorochrome-conjugated secondary antibody in 100 µL of 1X Staining Buffer and incubate for 15-30 minutes at 4°C in the dark.

14. Wash cells twice as in step 8.

15. Resuspend the cells in 200 uL 1X Staining Buffer and analyze on flow cytometer.