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Protocol

Flow Cytometry Phosphorylated Protein Fixation/Permeabilization Kit

This protocol was developed for analysis of 1x10^6 cells.

1. Bring all buffers to Working Temperature prior to use (see Kit Components Table).

2. Wash cells with PBS. Discard supernatant.

3. Add 0.5 mL Fixation Buffer. Mix well and incubate at RT for 15 min.

4. Centrifuge at 400-600 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 RT for 5 minutes.

6. Centrifuge at 400-600 g for 5 minutes and aspirate (do not dump) the supernatant.

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

8. Add 1.5 mL Staining Buffer (1X). Mix well and centrifuge at 400-600 g for 5 minutes and aspirate (do not pour off) the supernatant.

9. Repeat wash step 8.

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

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11. Stain cells with desired antibodies at pre-titrated concentrations.

12. After incubation, wash cells twice as in step 8.

13. If using secondary antibody, dilute the secondary antibody using Staining Buffer (1X).

Add 100 uL of diluted secondary antibody and incubate.

14. After incubation, wash cells twice as in step 8.

15. Resuspend cells in 200 uL Staining Buffer (1X).

16. Analyze sample with flow cytometer.

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