Intracellular Staining Using PFA and Saponin

Reagents:

1X PBS supplemented with 2% FBS, 0.02% NaN_3

10X PBS

Paraformaldehyde (PFA)

Saponin

Sodium carbonate

Distilled H_2O

Procedures:

1. Prepare PFA solution:

   a. Heat up to boiling 90 mL of H_2O in a microwave.
   b. Let temperature cool down.
   c. Add 4g of PFA when temperature reaches 68°C.
   d. Agitate with a magnetic stirrer at 50ºC[1] and add some droplets of sodium carbonate[2].
   e. Allow the PFA to undergo complete dissolution (it becomes totally transparent).
   f. Cool down the solution in ice.
   g. Add 10 mL of 10x PBS.
   h. Agitate with a magnetic stirrer until complete dissolution.
   i. Adjust pH to 7,3.
   j. Filter with a ø45 μm if necessary.
   k. Aliquote in 5 mL volume and freeze at –20°C[3].
   l. Dilute in 1x PBS to get a 2% concentration

2. Prepare PBS-saponin solution: 1X PBS, 5% FBS, 0,5% saponin. Dissolution of saponin should be done in agitation for 2 hours, in the dark, right before usage.

[1] Do not let temperature raise above 65ºC to avoid PFA degradation.
[2] PFA can only undergo complete dissolution at basic pH.
[3] 4% PFA solution has a short shelf life. Use freshly prepared or thawed PFA solution for your experiments.

3. Transfer lymphocytes to a 15-mL falcon tube or to a 96-well plate.

4. Sediment lymphocytes by centrifugation at 1400 rpm for 5 min and discard the supernatant.

5. Perform surface staining as usual or proceed for the next step.

Fixation step

6. Resuspend the pellet in 1 mL (tubes) or 150 μL (plate) 4% PFA.

7. Agitate for 5 minutes at room temperature.

8. Add 10 mL (tubes) or 150 μL (plate) PBS and sediment cells by centrifugation at 1400 rpm for 5 min.

9. Discard the supernatant and repeat step 5.

Permeabilization step

10. Add 5 mL (tubes) or 300 μL (plate) PBS-saponin.

11. Agitate for 10 minutes at room temperature.

12. Sediment cells by centrifugation at 1400 rpm for 5 min.

13. Add the intracellular antibodies diluted in PBS-saponin

14. Incubate for 30 minutes at 4ºC, in the dark.

15. Add 5 mL (tubes) or 300 μL (plate) PBS-saponin and sediment cells by centrifugation at 1400 rpm for 5 min.

16. Discard the supernatant and repeat step 5.

17. Discard the supernatant and resuspend cells is PBS.