biogems

Protocol for Intracellular Antigen Staining

The BioGems support buffers can be used to simultaneously investigate cell surface and intracellular antigens. The Transcription Factor Fixation/Permeabilization solution can be used with the relevant antibodies to stain for transcription factors such as Foxp3 and intranuclear staining of cytokines, chemokines, and nuclear proteins.

- 1. Generate fresh working solution of the Permeabilization buffer (cat# <u>92110-00</u>) by diluting the 10x concentrate to 1x with distilled water. Around 8.5 mL of the working solution is required for each sample.
- 2. Generate fresh working solution of the Transcription Factor Fixation/Permeabilization buffer by diluting one part of the concentrate solution (cat# <u>92550-00</u>) with 3 parts of the diluent (cat# <u>92160-00</u>). 1 mL of the working solution is required for each sample.
- 3. Arrange the sample of the cells of interest for Flow Cytometry analysis.
- 4. Optionally, utilize a relevant viability dye to exclude dead cells from analysis.
- 5. Utilize conjugated antibodies to stain the cell surface markers of interest.
- 6. Wash the pellet and discard the supernatant.
- 7. Dissociate the pellet by subjecting the sample to pulse vortex.
- 8. To each sample, add 1 ml of the Transcription Factor Fixation/Permeabilization working solution and pulse vortex.
- 9. Incubate the sample for at least 30 minutes in the dark at 4°C or room temperature.
- 10. To each sample, add 2mL of the 1x Permeabilization working solution.
- 11. For 5 minutes, centrifuge the samples at 300-400xg at room temperature.
- 12. Discard the supernatant and optionally repeat steps 10-12.
- 13. Resuspend the pellet in the residual volume of 1x Permeabilizaiton Buffer of about 100 µL.
- 14. Add the conjugated antibodies with intracellular antigen specificities to the samples.
- 15. For 30 minutes, incubate the samples in the dark at room temperature.
- 16. To each sample, add 2mL of the 1x Permeabilization working solution.
- 17. For 5 minutes, centrifuge the samples at 300-400xg at room temperature.
- 18. Discard the supernatant and repeat steps 16-18.
- 19. Resuspend the samples in the appropriate buffer to utilize in flow cytometry analysis.