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# 92110-00) 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# 92550-00) with 3 parts of the diluent (cat# 92160-00). 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.