MaxPar® Cytoplasmic Antigen Staining Protocol

Reagents and Materials:

- MaxPar® Metal Conjugated Antibodies
- MaxPar® Cell Staining Buffer (Cat# 201068)
- MaxPar® Fix and Perm Buffer (Cat# 201067)
- MaxPar® Water (Cat# 201069)
- MaxPar® Intercalator –Ir (Cat# 201192A [125 µM] or 201192B [500 µM])
- 16% Formaldehyde (Thermo Scientific Cat# 28908)
- Saponin (Sigma Cat# S7900)
- Polystyrene or Polypropylene Round-bottom Tubes, 5 mL capacity, 12 x 75 mm
- Polystyrene or Polypropylene Round-bottom Tubes with Cell-Strainer Cap, 5 mL capacity, 12 x 75 mm
- 1.5 mL microfuge tubes
- Pipet tips with aerosol barrier
- Centrifuge capable of holding 5 ml tubes
- Vacuum aspirator
- Vortexer
- [Optional] Cisplatin (Sigma Cat# 479306)
- [Optional] DMSO (ATCC Cat# 4-X)
- [Optional] Fc-receptor Blocking Solution (BD Cat# 553142 [mouse], BioLegend Cat# 422302 [human])

Important Notes Before Starting:

- This protocol should be followed for staining cytokines, and other secreted proteins, in addition to intracellular antigens that are localized outside of the nucleus (eg. IκBα, Cleaved Caspase-3). For phospho-protein staining, use the MaxPar Phospho-Protein staining protocol.

- For cell centrifugation steps, centrifugation should be performed for 5 minutes at 300g before cell fixation, and for 5 minutes at 800g after cell fixation. The increased centrifugation speed after cell fixation will result in greater cell recovery.

- DVS Sciences antibodies are pre-titrated and we recommend staining with 1 µl of each antibody for 3 million cells in a 100 µl staining volume; however antibodies can be titrated for individual experiments.

- An optional Fc-blocking step is recommended in the following protocol to prevent binding of MaxPar Metal Conjugated Antibodies to Fc receptors, which will result in high non-specific background signal. Fc receptors specific for IgG, including FcγR1 (CD64), FcγRII (CD32) and FcγRIII (CD16) are present on many cell types, with particularly high expression on myeloid, granulocyte and B cell lineages. Several antibody supply companies provide both human and mouse Fc-blocking reagents that can be used as indicated in the following protocol to minimize non-specific antibody binding.

- The following protocol recommends staining of surface markers at the same time as intracellular antigens, following cell fixation and permeabilization, which will result in detection of both surface and intracellular antigen fractions.

However, some surface marker epitopes may be degraded during the fixation and/or permeabilization steps, which should be evaluated for each antibody clone. In these cases, it is recommended to perform surface staining prior to the cell fixation and/or permeabilization, to achieve the best possible surface staining.

Reagents and Solutions to Prepare in Advance:

Permeabilization Buffer: prepare permeabilization buffer by adding saponin to a final concentration of 0.3% in MaxPar Cell Staining Buffer. For example, prepare 100 mL of permeabilization buffer by dissolving 300 mg of saponin in a final volume of 100 mL of Cell Staining Buffer.

Antibody Cocktail: prepare a cocktail of MaxPar Metal Conjugated Antibodies in permeabilization buffer. It is recommended to prepare antibody cocktail in a total volume of 50 µL, so that when added to 50 µL of cells the total staining volume is 100 µL. *The antibody cocktail can be stored for up to 24 hours before staining.*

Intercalation Solution: prepare 1 mL of cell intercalation solution for each sample by adding MaxPar Intercalator-Ir into MaxPar Fix and Perm Buffer to a final concentration of 125 nM (a 1000X dilution of the 125 µM stock solution) and mix by vortexing. For example, for 10 samples, prepare intercalation solution by adding 10 µL of 125 µM Intercalator-Ir to 10 ml of Fix and Perm Buffer.

[Optional] Cisplatin: prepare a 25 mM (1000X) stock solution of cisplatin by dissolving 7.5 mg of cisplatin in 1 mL of DMSO. Aliquot and freeze at -20˚C for long-term storage.

Protocol:

1. Prepare cells of interest from cell culture or primary tissue and activate cells by adding stimulus for appropriate length of time.

2. Following stimulation, transfer cells to a tube of appropriate size.

[Optional] Cisplatin Viability Stain:

   i. Spin cells, and discard supernatant by aspiration.
   ii. Wash cells with PBS, spin and discard supernatant by aspiration.
   iii. Resuspend cells to 1 X 10^7/ml in PBS and add cisplatin to a final concentration of 25 µM (1000X dilution of stock solution).
   iv. Incubate at room temperature for 5 minutes.
   v. Wash with Cell Staining Buffer using 5X the volume of the stained cell suspension, spin and discard supernatant by aspiration.
   vi. Resuspend cells to 1 X 10^7/ml in Cell Staining Buffer and proceed with the protocol.

3. Fix cells by adding Formaldehyde to a final concentration of 1.6% (10X dilution of the 16% stock solution) either directly to the cells in culture media (from step 2), or to cisplatin-stained cells (from step vi).

4. Mix gently and thoroughly and incubate for 10 minutes at room temperature.

5. Spin cells, and discard supernatant by aspiration.

6. Wash cells with 10 mL of permeabilization buffer, spin and discard supernatant.

7. Resuspend cells in permeabilization buffer and aliquot 3 million cells into 5 mL tubes for each sample to be stained.

8. [*Optional*] Block Fc-mediated antibody binding: add Fc-Receptor Blocking Solution to each tube, according to manufacturer's instruction, and incubate for 10 minutes at room temperature.

9. Without washing off Fc-Receptor Blocking Solution, add the antibody cocktail to each tube so the total staining volume is 100 µL.

10. Gently vortex samples and incubate for 30 minutes at room temperature.

11. Following the incubation, wash by adding 2 mL MaxPar Cell Staining Buffer to each tube, centrifuge and discard supernatant by aspiration.

12. Repeat for a total of two washes, and resuspend cells in residual volume by gently vortexing after final wash/aspiration.

13. Add 1 ml of the intercalation solution to each tube and gently vortex. Incubate for 1 hour at room temperature or leave overnight at 4°C. *Note: Cells can be left at 4°C in the intercalation solution up to 48 hours.*

14. Wash cells by adding 2 ml of MaxPar Cell Staining Buffer, centrifuge and discard supernatant by aspiration.

15. Repeat for a total of two washes with MaxPar Cell Staining Buffer.

16. Wash cells with 2 ml of MaxPar Water, centrifuge and discard supernatant by aspiration.

17. Leave cells pelleted until ready to run on CyTOF®. Immediately prior to CyTOF data acquisition, adjust cell concentration to 2.5-5 x 10^5/ml with MaxPar Water and filter cells into cell strainer cap tubes.

18. Acquire data on CyTOF.