Maxpar® Nuclear Antigen Staining

WARNING Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

Reagents and Materials

Included in Panel Kit

- Maxpar® Metal Conjugated Antibodies
- Maxpar® Cell Staining Buffer (Cat# 201068)
- Maxpar® Fix and Perm Buffer (Cat# 201067)
- Cell-ID™ Intercalator–Ir (Cat# 201192A [125 µM] or 201192B [500 µM])
- Maxpar® Water (Cat# 201069)
- Maxpar® Nuclear Antigen Staining Buffer Set (Cat# 201063)
    - Nuclear Antigen Staining Buffer Concentrate (4X)
    - Nuclear Antigen Staining Buffer Diluent
    - Nuclear Antigen Staining Perm (1X)

Optional

- Cell-ID™ Cisplatin (Cat# 201064)

Other Required Materials and Equipment

- 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
- Centrifuge capable of holding 5 mL tubes
- Vacuum aspirator
- Vortex
- [optional] Fc-Receptor Blocking reagent

Important Notes Before Starting

- This protocol is intended for staining antigens located within the nucleus, including transcription factors. For staining secreted proteins, including cytokines, or antigens located in the cytoplasm, follow the Maxpar Cytoplasmic/Secreted Antigen Staining Protocol. For staining phospho-proteins, follow the Maxpar Phosphoprotein Staining Protocol.
- This protocol is optimized for detection of antigens located within the nucleus; however the staining intensity of some surface markers may be diminished as a result of the fixation and permeabilization. Other protocols may enable adequate, although less optimal, detection of nuclear antigens, but allow for better preservation of surface staining.
- For cell pelleting steps, centrifugation should be performed for 5 minutes at 300 - 400g before cell fixation, and for 5 minutes at 600 - 800g after cell fixation. The increased centrifugation speed after cell fixation will result in greater cell recovery.
- Fluidigm 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 should 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 may 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.

Reagents and Solutions to Prepare in Advance

Maxpar® Nuclear Antigen Staining Buffer working solution:

Prepare fresh Nuclear Antigen Staining Buffer working solution by diluting the 4X Nuclear Antigen Staining Buffer Concentrate (1 part) with Nuclear Antigen Staining Buffer Diluent (3 parts). You will need 1 mL of the working solution for each sample. For example, if staining 10 samples, dilute 2.5 mL of the Nuclear Antigen Staining Buffer Concentrate with 7.5 mL of the Nuclear Antigen Staining Buffer Diluent.

Maxpar® Intercalation Solution:

Prepare 1 mL of cell intercalation solution for each sample by adding Cell-ID 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.

Protocol

*[**Optional]:** If not performing Cell-ID Cisplatin viability-stain, proceed to step #1 of the protocol below.]*

***Cell-ID Cisplatin Viability Stain.** Please see Cisplatin Technical Data Sheet for additional details about the use of this reagent. Detect Cell-ID Cisplatin in the 195Pt channel of the CyTOF mass cytometer.*

a. *Wash cells with PBS, centrifuge at 300-400xg for 5 minutes and discard supernatant by aspiration.*
b. *Resuspend cells to 1 X 10^7/ml in PBS and add Cell-ID™ Cisplatin to a final concentration of 5 µM (1000X dilution of 5 mM stock solution, ie. 1 µL Cell-ID Cisplatin added to 1 mL of cell suspension).*
c. *Mix well and incubate at room temperature for 5 minutes.*
d. *Quench staining with MaxPar® Cell Staining Buffer using 5X the volume of the cell suspension (ie. add 5 mL to 1 mL of cell suspension), centrifuge and discard supernatant by aspiration.*
e. *Wash cells again with Cell Staining Buffer, centrifuge and discard supernatant by aspiration.*
f. *Proceed with cell surface staining in step #1 below.*

1. Resuspend cells in Maxpar Cell Staining Buffer and aliquot 1 to 3 million cells, in a volume of 50 µL, into 5 mL tubes for each sample to be stained.
2. **[Optional]** Fc-Blocking: add Fc-Receptor Blocking Solution to each tube and incubate for 10 minutes at room temperature. Without washing off Fc-Receptor Blocking Solution, continue with protocol.
3. Add 50 µL of the surface antibody cocktail to each tube so the total staining volume is 100 µL. (50 µl of cell suspension + 50 µl antibody cocktail.)
4. Gently vortex samples and incubate for 30 minutes at room temperature.
5. Wash by adding 2 mL Maxpar Cell Staining Buffer to each tube, centrifuge (300-400g) and discard supernatant by aspiration.

6 Vortex sample to thoroughly disrupt the pellet. ***Note:*** *It is essential to thoroughly disrupt the pellet by vortexing before adding Nuclear Antigen Staining Buffer working solution at this step.*

7 Add 1 ml of the Nuclear Antigen Staining Buffer working solution to each sample followed by gentle vortex.

8 Incubate at room temperature for 30 minutes.

9 Wash cells with 2 ml of Nuclear Antigen Staining Perm, centrifuge (600–800g) and discard supernatant.

10 Repeat step 9 for a total of 2 washes.

11 Resuspend pellet in residual volume with gentle vortexing.

12 Add intracellular antibody cocktail (50 µL) directly to residual volume and stain for 30-45 minutes at RT.

13 Wash 2X with 2 mL Maxpar Cell Staining Buffer.

14 Vortex sample to disrupt cell pellet.

15 Add 1 ml of the intercalation solution to each tube and gently vortex.

16 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.*

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

18 Wash cells with 2 ml of Maxpar Water, centrifuge and discard supernatant by aspiration.

19 Repeat for a total of 2 washes with Maxpar Water.

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

21 Acquire data on mass cytometer.

For technical support visit fluidigm.com/support

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