Imaging Mass Cytometry Staining Protocol for Frozen Tissue Sections

Hyperion Imaging System

Required Equipment

Wear protective nitrile powder-free gloves, safety glasses, and a laboratory coat when handling all materials and chemicals.

Required Equipment
Hyperion™ Imaging System
Centrifuge
Fume hood
Slide holder
Glass Coplin jar
Pipette 1,000 µL, 200 µL, 20 µL,
Hydration chamber
Orbital shaker plate
PAP pen
–80 °C, –20 °C, 4 °C refrigerator

Required Reagents

Product Name	Catalog Number	Manufacturer	Quantity
16% Paraformaldehyde	15710	Electron Microscopy Solutions	10 mL
Triton™ X-100	85111	Thermo Scientific™	100 µL per 5–7 slides
Maxpar® PBS	201058	Fluidigm	250 mL per 5–7 slides
Cell-ID™ Intercalator-Ir	201192A	Fluidigm	300–500 µL per slide (1:400 dilution)
Bovine Serum Albumin (BSA)	A3059	Sigma-Aldrich	10 g per 100 mL PBS

Product Name	Catalog Number	Manufacturer	Quantity
Maxpar Water	201069	Fluidigm	150 mL per 5–7 slides
Maxpar antibodies	Multiple	Fluidigm	Assay-dependent

Workflow

1 Fixation	2 Blocking	3 Antibody Staining	4 Nuclear Staining
Fix with 4% PFA	Block with 3% BSA.	Dilute antibodies in PBS/BSA, stain overnight	Iridium intercalator stain

Procedure

Staining for tissue imaging on the Hyperion Imaging System is similar to standard immunohistochemistry techniques in terms of tissue collection, tissue processing, and staining.

NOTE This protocol is used to test all pathologist-verified Maxpar antibodies for Imaging Mass Cytometry™ (IMC™) available for frozen tissue imaging, but it may require optimization for some antibodies and/or tissue types. For more detailed support, contact Fluidigm Tech Support at techsupport.fluidigm.com/.

1. Chill the slide holder (glass jar) to –20 °C for 1 hr or overnight
2. Transfer slides from the –80 °C refrigerator to chilled glass jar in the –20 °C refrigerator and allow to equilibrate for 1 hr.
3. Under the fume hood, use ampule cutter to open 16% PFA glass ampule. Discard broken glass appropriately in a hazardous sharps bin.
4. In a 50 mL tube, dilute 16% PFA to 4% PFA with PBS: Add 10 mL 16% PFA into 30 mL PBS.

NOTE Before dilution, 16% PFA and PBS should be pre-chilled in the 4 °C refrigerator for 45 min to 1 hr.

5. Transfer 40 mL 4% PFA solution into a plastic jar and store in the 4 °C refrigerator.
6. Place the glass jar with slides in the 4°C refrigerator for 5–10 min.
7. Transfer slides from the glass jar to the plastic jar with 4% PFA. Fix with 4% PFA for 30 min at 4°C.
8. Rinse slides 3 times in PBS, 5 min each at room temperature (RT).
9. Use With a PAP pen, circle the sample, using a Kimwipe® to assist in the process.

10 Block with 3% BSA for 45 min at RT.

11 Prepare the antibody cocktail. Calculate the total volume of antibodies at concentrations specific for the assay, and bring the volume up to a final volume of 0.5% BSA in Maxpar PBS. Place the slides in a hydration chamber and pipette the antibody master mix onto the section.

NOTE Vortex and centrifuge individual antibodies. Centrifuge the completed antibody cocktail at 13,000 x *g* for 3 min and pipet from the top of the tube to avoid antibody aggregates.

12 Stain with the antibody cocktail for 1 hr in a hydration chamber at RT.

13 Wash the slides in 0.2% Triton X-100 in Maxpar PBS for 8 min with slow agitation in Coplin jars. Repeat.

14 Stain the tissue with Intercalator-Ir in Maxpar PBS (300–500 µL/section for a 20 mm^2 section of 1:400 solution) for 30 min at RT in a hydration chamber.

15 Rinse 2 times in double-distilled water for 5 min each time.

16 Air-dry the slide for at least 20 min at RT.

NOTE

- Blocking solution should be diluted from 10% BSA freshly made from powder. The remaining 10% BSA should be aliquoted and stored at −20 °C, and then diluted at the time of use.
- Use enough blocking solution to cover the section (around 300–500 µL for a 20 mm^2 section).
- When using Fluidigm pathologist-verified Maxpar antibodies for frozen tissue imaging, consult the technical data sheets for the recommended dilution ranges for individual antibodies.
- Add a small volume of individual antibodies into a larger volume of 0.5% BSA in Maxpar PBS diluent.
- BSA should be 0.5% concentration in the final antibody cocktail.
- The final volume of antibody cocktail needed depends on the size and location of your tissue sections and the number of slides. Determine volume empirically.

IMPORTANT It is recommended that you store the antibody cocktail on ice and add it to your samples within 1–2 hr of preparation for best results.

Tips and Tricks

Fixation

- Store 16% PFA away from direct light.
- Prepare 4% PFA freshly in PBS each time before staining.

Antibody Staining

- Optimize each antibody concentration by performing a titration series on the relevant tissue type. Refer to the Fluidigm technical data sheets for recommended starting concentrations.
- Always refer to the Fluidigm technical data sheet to get an idea of the localization and expected staining pattern of the antigen prior to staining. Other recommended sources for expected staining behavior data on a broader array of tissues include the published literature, antibody vendor data sheets, and proteinatlas.org/.
- Tween™ 20 can be used as a gentler alternative to Triton X-100 for washing slides if marker signal intensity is low. Using Tween-20 instead of Triton X-100 may also be preferable for staining some membrane markers.

Intercalator Staining

- The efficiency of intercalator staining can vary depending on the tissue origin and processing. Fluidigm recommends that you optimize intercalator concentration for specific tissues of interest by performing a titration series.

For technical support visit techsupport.fluidigm.com. | For general support visit fluidigm.com/support.

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