Imaging Mass Cytometry Staining Protocol for FFPE 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
Heating block (96 °C)
Slide oven (60 °C)
Orbital shaker plate
PAP pen

Required Reagents

Product Name	Catalog Number	Manufacturer	Quantity
M-xylene ReagentPlus®	185566-1L	Sigma-Aldrich®	50 mL per 5–7 slides
Anhydrous ethyl alcohol	676829	Sigma-Aldrich	172.5 mL per 5–7 slides
Antigen Retrieval Reagent pH 9 (10x)	S236784-2	Agilent®	4 mL per 2 slides
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
Maxpar Water	201069	Fluidigm	150 mL per 5–7 slides
Maxpar antibodies	Multiple	Fluidigm	Assay-dependent

Workflow

1 Dewaxing	2 Hydration	3 Antigen Retrieval (AR)	4 Blocking	5 Primary Incubation	6 Secondary Incubation
Dewax in xylene.	Hydrate in ethanol.	Incubate in AR buffer.	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™), but it may not be optimized for all antibodies or tissue types. For more detailed support, contact Fluidigm Tech Support at techsupport.fluidigm.com/.

1. Bake the slides for 2 hours at 60 °C in the slide oven/dryer. Alternatively, if no oven is available, a heat block set at 60 C can be used. In either case, ensure that all visible wax is removed.
2. Turn the heating block on to 96 °C during the baking step.

IMPORTANT Calibrate your heating block temperature to make sure that the antigen retrieval solution is at 96 °C.

3. Prior to dewaxing, prepare 50 mL conical tubes containing 40 mL of antigen retrieval solution (10X diluted to 1X) and put the tubes in the heating block (96 °C) with loose lids.

NOTE Two slides placed back-to-back can fit into one 50 mL conical tube.

4. Dewax the slides in fresh xylene in the fume hood for 20 minutes with loose lids.
5. Hydrate the slides in descending grades of ethanol (100%, 95%, 80%, 70%), 5 minutes each.

   NOTE Some antibodies may require different antigen retrieval conditions for optimal performance on different tissues. Optimize these conditions for the combination of antibodies in the panel. Fluidigm antibodies are optimized to basic (pH 9) antigen retrieval conditions when used in combinatorial panels.

6. Wash the slides in Maxpar Water for 5 minutes in a Coplin jar placed on an orbital shaker plate with gentle agitation.
7. Insert the slides with tissues into preheated antigen retrieval solution and incubate them for 30 minutes, leaving the lids loose.

8 Following incubation, remove the slides from the heating block, place the tubes containing the antigen retrieval solution and slides on a lab bench, and cool them to 70° C by monitoring the temperature of the AR solution for about 10 minutes.

9 Wash the slides with Maxpar Water for 10 minutes in a Coplin jar with gentle agitation (orbital shaker).

10 Wash the slides with Maxpar PBS for 10 minutes with gentle agitation.

11 Use a PAP pen to encircle the sample.

12 Block with 3% BSA in Maxpar PBS for 45 minutes at room temperature in a hydration chamber. (You can use an empty pipette tip box where the slides rest on the tip shelf and the bottom is filled halfway with water).

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).

13 To 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

- When using Fluidigm pathologist-verified Maxpar antibodies for imaging, consult the technical data sheets for the recommended dilution ranges for individual antibodies.
- Spin the antibody at 13,000 *x g* for 2 minutes and pipet from the top of the tube to avoid antibody aggregates.
- Add a small volume of individual antibodies into a larger volume of 3% 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 hours of preparation for best results.

14 Incubate overnight with the antibody cocktail at 4 ˚C in a hydration chamber. (See Step 12)

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

16 Wash the slides in Maxpar PBS for 8 minutes with gentle agitation. Repeat.

17 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 minutes at room temperature in a hydration chamber.

IMPORTANT It is recommended that users optimize intercalator concentration for tissues of interest.

18 Wash the slides in Maxpar Water for 5 minutes with gentle agitation.

19 Air-dry the slides for at least 20 minutes at room temperature.

Tips and Tricks

IMPORTANT It is recommended that you begin by optimizing the staining protocol using smaller panels (approximately 3–5 markers) on positive and negative control tissue sections.

Dewaxing and Hydration

- Try to use fresh-cut tissue sections, because pre-cut tissue integrity may degrade over time.
- Use fresh xylene to dewax your samples before staining. If you notice residual wax after baking, consider doing two 10-minute dewaxing steps with fresh xylene.

Pre-Staining

Substituting Maxpar Water with TBS (for phosphorylated targets) or Maxpar PBS before staining might enhance the signal for some clones under some circumstances. Test this substitution empirically first, with appropriate controls.

Antigen Retrieval

- Heat-induced epitope retrieval (HIER) according to the protocol is recommended for our catalog of IMC-verified antibodies, because all of our antibodies have been tested using this protocol. However, the optimal protocol for any combination of antibodies and tissues might vary from the standard, and your lab should test your protocol empirically.
- Proteolytic-induced epitope retrieval (PIER) may be required for some targets. PIER uses enzymes such as proteinase K, trypsin, and pepsin to degrade the peptides masking the epitope. Check the literature to determine if PIER is recommended for your antibody of interest. Fluidigm has not tested PIER methods for our catalog of IMC-verified antibodies.
- Refer to existing literature on immunohistochemistry (IHC) staining of your clone of interest to determine the recommended staining protocols for your tissue of choice.
- Some antibodies prefer acidic or neutral pH retrieval conditions. Empirically determine and test pH for all antibodies in the panel. Fluidigm antibodies for imaging are all verified in basic (pH 9) antigen retrieval conditions.
- In addition to antigen retrieval solution, other parameters that can be optimized are temperature and duration of the retrieval.

Antibody Staining

- Some antibody clones may be more appropriate for use on frozen sections than on FFPE tissue. You may need to look for an alternative clone that works for the specific type of section you are working with.
- 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/.
- Fluidigm recommends an overnight incubation at 4 °C of your slides with the antibody staining cocktail. If nonspecific binding results are high, we recommend that you shorten the incubation time, but a shortened time may not be beneficial for all antibodies in your panel.
- 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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