FFPE MIBI Staining Protocol

Tissue Staining for Multiplexed Ion Beam Imaging

FFPE MIBI Staining Protocol utilizes PT Module method for Heat-Induced Epitope Retrieval (HIER)

SAFETY: The solutions listed in this SOP can be corrosive to tissues and can cause skin damage. They are harmful if swallowed or inhaled. Avoid contact with eyes, skin, or clothing. Wear eye protection, gloves and protective clothing when handling. Be aware of safety precautions relating to the handling and use of all solutions. Consult the product labeling or Safety Data Sheet (SDS), as necessary.

Equipment, Materials, and Reagents

Equipment	Slide Oven or Incubator Thermo Scientific PT Module Heat-resistant slide chamber Pap Pen Moisture chamber Slide staining jar Microcentrifuge
Materials	MIBIslide (Ionpath Cat. #567001) Ultrafree-MC Spin Filter, 0.5mL 0.1µm 0.2 µm Rapid-Flow Sterile Disposable Filter Units Aerosol Barrier Pipette Tips
Reagents	Xylene 100% Reagent Alcohol 95% Reagent Alcohol 80% Reagent Alcohol 70% Reagent Alcohol Heat-induced epitope retrieval (HIER) solution: 10X Tris with EDTA, pH 9 20X TBS Wash Buffer with Tween 20 (TBS-T) (Ionpath Cat. #567005) Donkey serum 10X Low-barium PBS pH 7.4 (Ionpath Cat. #567004) 10X Tris pH 8.5 (Ionpath Cat. #567003) MIBI diH_2O (Ionpath Cat. #567002) Glutaraldehyde, 70% EM Grade PBS tablets for HIER using PT module method

General Notes:

- Always try to limit mechanical damage to the tissue and slide surface.
- Avoid introducing background by using reagents free of metals.
- Once samples have been rehydrated, do not let them dry out until instructed to do so at the end.
- Do not store reagents in glassware as this may result in metal contamination.

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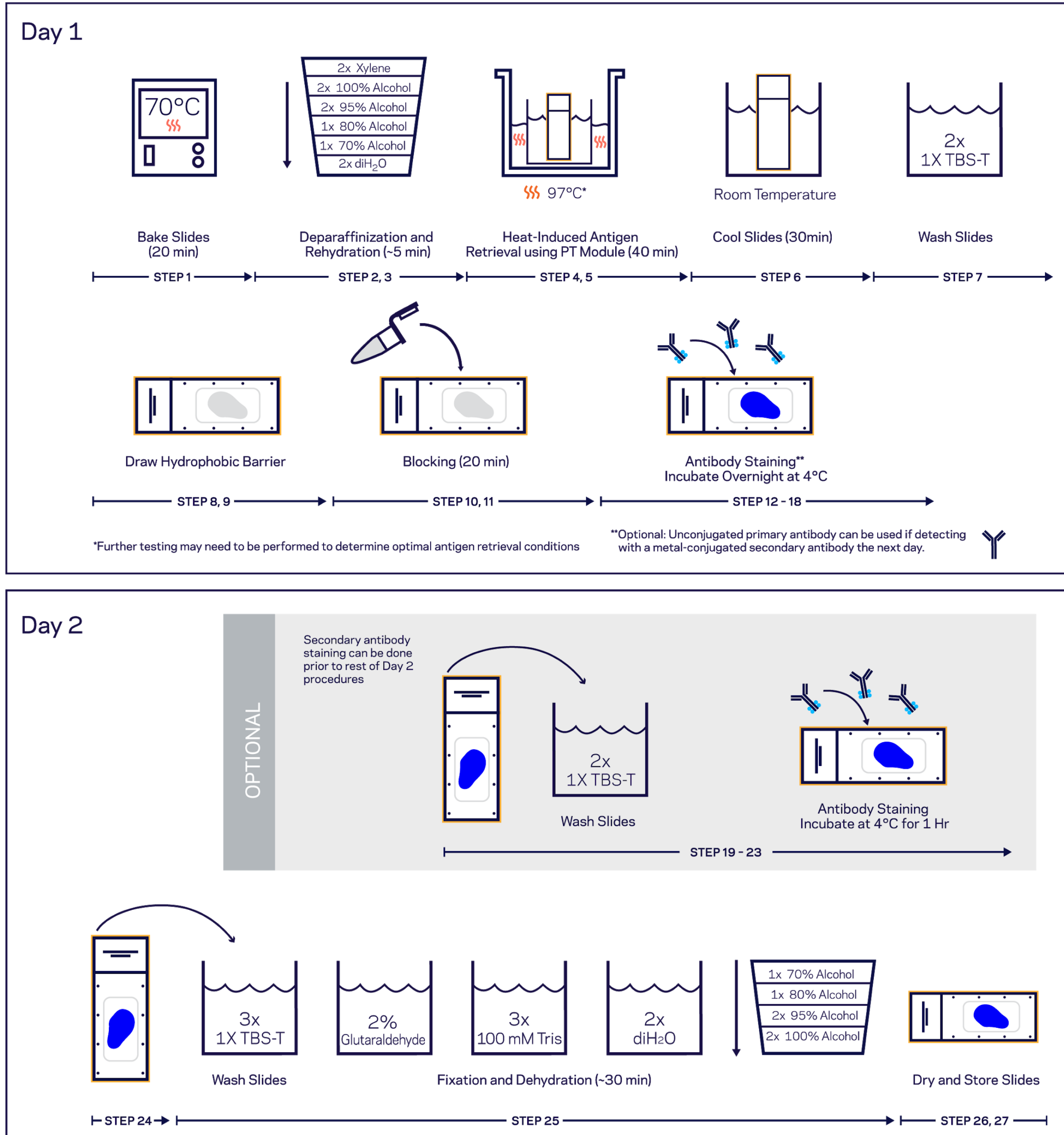
Figure 1: Staining Overview



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Day 1

Prepare Reagents

- Make 1 L 1X TBS-T by diluting 50 mL of 20X TBS-T into 950 mL of diH_2O.
- Make 1X HIER solution by diluting 10X Tris with EDTA antigen retrieval buffer 1:10 in diH_2O.
- Prepare blocking buffer by diluting donkey serum in 1X TBS-T to a final concentration of 5% (e.g., To make 1 mL of blocking buffer, dilute 50 µL of donkey serum in 950 µL of 1X TBS-T), store in 4°C until ready to use.

Prepare Heat-induced Epitope Retrieval

PT Module method: *

- Make 1.4 L PBS following manufacturer's instructions.
- Fill one tank with 1.4 L PBS and place the prepared slide chamber containing 1X HIER buffer into the tank.
- Preheat the tank in PT Module to 75°C

* For other methods, further testing may need to be performed to determine optimal antigen retrieval conditions.

Deparaffinization and Rehydration

1. Place the slides in a slide rack and bake at 70°C for 20 minutes in an incubator or oven.
2. Remove the slides from heat and wash the slides with light to moderate agitation in the following buffers:
   - 2.1 Xylene 2 times for 30 seconds each
   - 2.2 100% Reagent alcohol 2 times for 30 seconds each
   - 2.3 95% Reagent alcohol 2 times for 30 seconds each
   - 2.4 80% Reagent alcohol for 30 seconds
   - 2.5 70% Reagent alcohol for 30 seconds
   - 2.6 diH_2O 2 times, for 30 seconds each
3. Keep the slides in a fresh diH_2O container until ready for antigen retrieval. **

** From this point forward, DO NOT let slides dry out until instructed to do so at the end of the procedure.

Heat-Induced Epitope Retrieval

4. Place slides in preheated slide chamber containing 1X HIER buffer inside the PT Module.
5. Run the PT Module at 97°C for 40 min. After 40 min, PT Module will automatically cool to 65°C.
6. Once 65°C is reached, remove slides from PT Module and let it rest at room temperature for 30 min.

Antibody Blocking

7. Wash slides by dipping in 1X TBS-T for 2 min. Repeat in fresh 1X TBS-T for a second wash.
8. Draw solid PAP pen border around the tissue section and allow the barrier to dry for 15-30 seconds.

   Tip: To avoid potential leaks, do not press the pen down too hard while drawing the barrier. Take care to prevent the Pap Pen fluid from touching the tissue section.
9. Dip the slide briefly back in TBS-T to remove any PAP Pen residue.
10. Carefully remove TBS-T by tipping slide on its side and gently tapping the edge against a kimwipe.
11. Incubate the tissue area with 100 – 200 µL blocking buffer for 20 min at room temperature in a moisture chamber. Leave tissue incubated in blocking buffer until antibody master mix is ready to be added.

Antibody Panel Preparation

12. Prepare 100 – 200 µL of the antibody panel per tissue area to be stained.

    12.1 To prepare your own antibody panel:
    - Spin down all antibody tubes at 10,000 xg for 5 minutes. Do not disturb the bottoms of the antibody tubes when pipetting.
    - Prepare antibody master mix using blocking buffer as the antibody diluent.

    12.2 To prepare a panel using an Ionpath lyophilized panel:
    - Reconstitute the lyophilized panel at a concentration of 100 µL of blocking buffer per test.
13. Filter the antibody panel by first pre-wetting a 0.1 µm Ultrafree-MC Spin Filter, 0.5 mL, with 100 µL of blocking buffer. Spin at 10,000 xg for 2 minutes and remove blocking buffer flow-through with a pipette.
14. Transfer antibody panel into spin column and spin at 10,000 xg for 2 minutes.
15. Discard spin column; use the flow-through as the filtered antibody panel.

Antibody Staining

16. Carefully remove blocking buffer by tipping slide on its side and gently tapping the edge against a kimwipe.
17. Place slides in a moisture chamber and add 100–200 µL of antibody master mix to tissue, taking care to avoid contact with tissue or creation of air bubbles.
18. Close the lid and incubate the slides in the moisture chamber overnight at 4°C (in the dark if fluorophore-labeled antibodies are used).

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Day 2

Prepare Reagents

- Make 1X PBS by diluting 100 mL of 10X Low-Barium PBS pH 7.4 in 900 mL diH_2O.
- Prepare a working stock solution of 2% glutaraldehyde in low-barium PBS.
- Filter diH_2O and 2% Glutaraldehyde in PBS using 0.2 μm filtration units.
- Make 1X Tris by diluting 100 mL 10X Tris pH 8.5 in 900 mL filtered diH_2O.

Secondary Antibody Staining (If Applicable)

19. If using secondary antibodies, prepare secondary antibody panel following steps 12 – 15, else skip to step 24.
20. Carefully remove primary antibody panel by tipping slide on its side and gently tapping the edge against a kimwipe.
21. Wash slides by dipping in 1X TBS-T for 2 min. Repeat in fresh 1X TBS-T for a second wash.
22. Place slides in a moisture chamber and add 100 – 200 μL of secondary antibody panel to tissue, taking care to avoid contact with tissue or creation of air bubbles.
23. Close the lid and incubate the slides in the moisture chamber at 4°C for 1hr (in the dark if fluorophore-labeled antibodies are used).

Fixation and Dehydration

24. Carefully remove antibody master mix by tipping slide on its side and gently tapping the edge against a kimwipe.
25. Wash slides with light to moderate agitation in the following buffers:
    - 25.1 1X TBS-T, 3 times for 5 minutes each
    - 25.2 Filtered 2% Glutaraldehyde for 5 minutes
    - 25.3 Filtered 1X Tris pH 8.5, 3 times for 30 seconds each
    - 25.4 Filtered diH_2O, twice for 30 seconds each
    - 25.5 70% Alcohol for 30 seconds
    - 25.6 80% Alcohol for 30 seconds
    - 25.7 95% Alcohol twice for 30 seconds each
    - 25.8 100% Alcohol twice for 30 seconds each
26. Gently tap the edge of the slide against a kimwipe to remove excess alcohol and allow residual alcohol to evaporate at room temperature (approximately 5-10 min).
27. Dry slides in a desiccator for at least 1 hour prior to MIBIscope analysis or store slides in a vacuum chamber for long-term storage.

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