STANDARD OPERATING PROCEDURE

Title: Staining FFPE tissue sections for the Imaging Mass Cytometry (IMC) application

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1. PURPOSE

This SOP describes the procedure for staining the Formalin Fixed Paraffin Embedded (FFPE) tissue slides with metal-labeled antibodies for Standard BioTools Hyperion Imaging Mass Cytometry (IMC).

2. SCOPE

This SOP provides information for processing FFPE tissue specimens for IMC staining. It includes steps such as deparaffinization, hydration, antigen retrieval, blocking, and staining FFPE tissue with metal-labeled antibodies followed by DNA intercalator and preparing slides for laser ablation on the Hyperion.

This SOP may be used in conjunction with the SOP titled, “Lanthanide Antibody Labeling using Standard BioTools Maxpar Multiple Metal Labeling Kit.”

3. RESPONSIBILITIES

It is the responsibility of the person(s) performing this procedure to be familiar with lab safety procedures and to have basic laboratory skills. It is the responsibility of the analyst to follow the procedure steps as written and to document any deviations, problems, and observations during an experiment in their laboratory notebook.

4. EQUIPMENT

4.1. Chemical fume hood, NuAire Class II B2, NU-435-600, or equivalent

4.2. Vortex, Fisher Scientific, Analog Vortex Mixer 02215365, or equivalent

4.3. Mini centrifuge, Fisher Scientific, SPROUT, or equivalent

4.4. 4°C refrigerator, Fisher Scientific, ISOTEMP 97-920-1, or equivalent

4.5. -20°C freezer, Fisher Scientific, ISOTEMP 97-926-1, or equivalent

4.6. Decloaking chamber, BioCare Medical, DC-2012, or equivalent

4.7. Hot plate, VWR, 4x4 CER HOT/STIR 120V Standard, or equivalent

4.8. -80°C freezer, New Brunswick, Inova U101, or equivalent

4.9. Micropipettes, Rainin, Pipet-Lite XLS, or equivalent

4.10. pH meter, VWR, SymPHony SR01, or equivalent

4.11. Orbital shaker plate, Scientific Industries, Mini 100 Orbital Genie, or equivalent

4.12. Water unit, Millipore Sigma, MilliQ IQ7005, or equivalent

4.13. PAP pen, Vector, Immedge Pen H-4000, or equivalent

4.14. Hazardous chemical container

4.15. Vertical Slide Holder, Optimal Scientific, 3028, or equivalent

4.16. Hydration Chamber, Thomas Scientific, 1185U36, or equivalent

5. CHEMICALS

5.1. FFPE slides for IMC staining

5.2. 100% Xylene histological grade, Fisher Chemical, Catalog # X3P-1GAL or equivalent

5.3. 100% Ethanol molecular biology grade, Fisher Biochemicals, Catalog # BP2818-4, or equivalent

5.4. DAKO target retrieval solution pH 9.0 (10X), Dako, S2367, Fisher Bioreagents, Catalog # BP399-500, or equivalent

5.5. 10X PBS, Fisher Bioreagents, Catalog # BP399-500, or equivalent

5.6. BSA Powder, Sigma, Catalog # A3059- 50G, or equivalent

5.7. Ultrapure water Millipore Sigma, Milli-Q® IQ Catalog # 70XX or equivalent

5.8. Standard BioTools Cell Segmentation Kit, Standard BioTools, Catalog # TIS-00001

5.9. Cell-ID Intercalator ID (125 µM), Standard BioTools, Catalog # 201192B

6. REAGENTS

6.1. 95% Ethanol – make using 100% Ethanol molecular biology grade

6.1.1. Combine 285 mL 100% Ethanol + 15mL ultrapure water

6.1.2. Store in secondary container located in fume hood. Solution should be made fresh monthly or sooner if contamination has occurred, or debris is present in the container.

6.2. 80% Ethanol – make using 100% Ethanol molecular biology grade

6.2.1. Combine 240 mL 100% Ethanol + 60 mL ultrapure water

6.2.2. Store in green chemical staining containers in fume hood. Solution should be made fresh monthly or sooner if contamination has occurred or debris is present in the container.

6.3. 70% Ethanol – make using 100% Ethanol molecular biology grade

6.3.1. Combine 210 mL 100% Ethanol + 90 mL ultrapure water

Note: Store in green chemical staining containers located in fume hood. Solution should be made fresh monthly or sooner if contamination has occurred or debris is present in the container.

6.4. 1X PBS pH 7.4 ± 0.02

6.4.1. Prepare by adding 100 mL 10X PBS to 900 mL ultrapure water.

6.4.2. Store solution at RT for up to one month.

6.5. 3% Bovine Serum Albumin (BSA)

6.5.1. Weigh 3g ± 0.2g of BSA into a 250-ml beaker containing a stir bar.

6.5.2. Add 90 mL of 1x PBS pH 7.4 and stir until completely dissolved.

6.5.3. Transfer to a 100 mL graduated cylinder and bring final volume to 100 ml using 1x PBS pH 7.4.

6.5.4. Make 8mL aliquots and store aliquots at -20°C for up to one year. The "in use" vial is stored at 4°C for one month.

6.6. 0.5% BSA

6.6.1. Prepare 1000 µL in 1X PBS pH 7.4 by adding 167 µL 3% BSA to 833 µL 1X PBS.

6.6.2. Prepare fresh for each experiment.

6.7. Cell-ID Intercalator-Ir is stored in ready to use aliquots in the -20°C freezer.

6.7.1. Prepare fresh for each experiment as follows:

6.7.1.1. 125µM: 996µL of 1X PBS + 4µL intercalator

6.8. Metal Labeled Antibodies (Prepared in house or purchased through a supplier, such as Standard BioTools.)

7. PROCEDURE

Day 1

7.1. Baking the Slides

7.1.1. Set the heat block to 96°C and allow to warm up before use.

Note: The heat block will beep when the desired temperature has been reached.

7.1.2. Place FFPE slide(s) on the heat block and bake for 30 minutes to remove paraffin wax.

7.2. Decloaking Chamber Preparation

7.2.1. Fill the chamber with 500 mL ultrapure water.

7.2.2. Fill the two outside slide containers with 250 mL ultrapure water.

7.2.3. Fill the middle slide container with a mixture of 225 mL of ultrapure water and 25mL of DAKO target retrieval solution

7.2.4. Close the lid and ensure it is locked in place.

7.3. Dewaxing in Xylene

Note: Dewaxing with xylene and hydration with ethanol must be performed in the chemical fume hood. Xylene waste must be disposed of in the hazardous chemical container and NOT down the drain. Check the levels of xylene and ethanol in each chamber containers before use. Change monthly or if dirty/contaminated. Cover ethanol containers with paraffin to keep them from evaporating.

7.3.1. Put the slide(s) in a vertical slide holder and transfer to the chemical fume food. Add 100% xylene to two containers in the chemical fume hood. Dip the slide(s) 1x in the first 100% xylene container then leave in the container for 10 minutes with a loose lid. Repeat step using second 100% xylene container.

Note: If tissue is fragile, dipping the slide prior to leaving in the xylene container is not performed.

7.4. Hydrating in Ethanol

7.4.1. Hydrate the slide(s) in descending grades of ethanol (100%, 95%, 80%, and 70%) using designated containers in the chemical fume hood.

7.4.2. For each ethanol wash, dip the slide(s) 20x then leave in the container for 3 minutes.

7.4.3. Repeat step 7.4.2 two times for each grade of ethanol.

7.4.4. After putting the slide(s) into the first 80% ethanol container for the 3-minute incubation, turn on the decloaking chamber and start the program.

7.4.5. After the last 70% ethanol wash, dip the slide(s) in ultrapure water 20 times then leave in the water container for at least 5 minutes.

7.4.6. Leave slide(s) in water until decloaking chamber is ready, then transfer to decloaking chamber container. Do not let the slide(s) dry out.

7.5. Antigen Retrieval

7.5.1. Load slide(s) into the middle slide container once the decloaking chamber has reached 95°C.

Note: You will have 2 minutes to put the slides in before the program will automatically resume. Confirm the lid is locked and secure.

7.5.2. Following incubation, carefully remove the slide container from the chamber and place slide container on the benchtop to cool (~ 10 minutes).

7.5.3. Drain half of the DAKO target retrieval solution and replace with ultrapure water, then allow to sit for ~ 5 minutes until the slide container is cool to the touch.

7.5.4. Drain the water from the outside slide containers, as well as the decloaking chamber. Leave the decloaking chamber open overnight to allow it to dry completely.

7.6. Blocking with 3% BSA

7.7. **Note:** Allow 3% BSA to acclimate to room temperature before use.

Note: All ultrapure water and 1X PBS washes should be performed on the shaker (4-4.5 setting) unless otherwise stated.

7.7.1. Wash the slide(s) (from Step 7.5.2) in ultrapure water for 5 minutes on the shaker in a new container.

7.7.2. Wash the slide(s) in 1X PBS for 5 minutes on the shaker.

7.7.3. Carefully dry the slide(s) with a Kimwipe and use a PAP pen to draw a circle around the tissue(s).

7.7.4. Add enough 3% BSA to cover the entire tissue area.

7.7.5. Place slide(s) in a hydration chamber and incubate for 45 minutes at room temperature on the shaker.

7.8. Making the Antibody Cocktail and Primary Incubation

7.8.1. Prepare antibody cocktail in 0.5% BSA.

7.8.1.1. Calculate how much of each antibody you need based on the dilution and the total volume of antibody cocktail required for your slide(s). This should be the same volume used for blocking the slide(s) with 3% BSA.

7.8.2. Vortex and spin down all antibodies before use. Keep all antibodies on ice.

7.8.3. Prepare an intermediate antibody dilution as required depending on volumes.

7.8.4. Add small volume of individual antibodies to larger volume of 0.5% BSA.

7.8.5. After the blocking incubation, (from Step 7.6) decant and use a Kimwipe to carefully dry around the outside of the PAP pen circle.

7.8.6. Add the antibody cocktail slowly over the tissue for each slide(s). Make sure the entire tissue is covered.

7.8.7. Place the slide(s) in a hydration chamber and add ultrapure water to the bottom.

7.8.8. Refrigerate at 4°C overnight.

Day 2

7.9. Secondary Incubation with Intercalator

Note: All ultrapure water and 1X PBS washes should be performed on the shaker (4-4.5 setting) unless otherwise stated.

7.9.1. Decant the antibody solution from the slide and wash the slide(s) in 1X PBS for 5 minutes on the shaker. Repeat this step twice.

7.9.2. Stain tissue with Cell-ID Intercalator-Ir and incubate for 30 minutes at room temperature on the shaker.

7.10. Wash the slide(s) in 1X PBS for 5 minutes on the shaker at room temperature.

7.11. Wash the slide(s) in ultrapure water for 5 minutes on the shaker.

7.12. Dry slide(s) using a Kimwipe and air from the chemical fume hood. Do not blow the air too close to the tissue.

7.13. Slide is ready to ablate either same day or place in designated storage area.

8. VERSION HISTORY

8.1. New SOP.