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Imaging Mass Cytometry Antibody Staining

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1 Works for me dx.doi.org/10.17504/protocols.io.bfz8jp9w

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ABSTRACT

The following SOP describes procedures required for the antibody staining of formalin fixed, paraffin-embedded (FFPE) samples. In this SOP, the term “samples” refers to FFPE tissue samples.

GUIDELINES

FFPE: Formalin-fixed, paraffin-embedded.
Slide: Refers to a microscopy glass slide containing one or multiple Samples.
Sample: Refers to either a clinical FFPE tissue section from a patient or a cell pellet cytoblock FFPE control sample on a glass slide.
Unstained sample: Refers to a sample prior to antibody staining.
Stained sample: Refers to a sample after antibody staining.
Antibody panel A pre-defined mix of antibodies used for staining.

MATERIALS

NAME	CATALOG #	VENDOR
Deionized Water		
UltraClear Clearing Reagent	7104-3905.5000PE	
Horse Serum		Gibco, ThermoFisher
Bovine Serum Albumin	A3059-500G	Sigma Aldrich
Ethanol Absolute 2.5% IPA	7002103	
TBS pH 7.6 (2.42 g Trizma 8g NaCl 1L dH2O)		
TBS-T pH 7.6 (1L TBS 1mL Tween (0.1%))		
HIER Buffer pH 9.2	S237584-2	Dako
3% BSA in TBS-T(0.1%)		
6% BSA in TBS-T(0.2%)		
Metal Conjugated Antibodies diluted in blocking solution		
Cell-ID Intercalator-Ir 500uM	201192B	Fluidigm
Ir-Intercalator Solution (1 µM Cell-ID™ Intercalator-Ir diluted in TBS)		
Decloaking Chamber™ NxGen	B3-DC2012	
Path Histo AS-2		
Cellstain 6		
Hydrophobic Barrier PAP Pen ImmEdge	H-4000	Vector Laboratories
Wet-Chamber	HL97.1	Carl Roth
1.5 mL reaction tubes	72.690.001	Sarstedt
15 mL reaction tubes		

SAFETY WARNINGS

Sample de-parafinization and rehydration must be performed in a fume hood.

BEFORE STARTING

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Conjugated antibodies are first validated using imaging mass cytometry to confirm successful conjugation and sufficient signal intensity. The images are visually inspected for expected staining patterns, co-localisation subcellular localisation of the marker. Antibodies can then be used in final sample staining once these validation checks have been passed.

Sample de-paraffinization and rehydration of samples

1 1. Unstained samples should have time to equilibrate to room temperature for at least 10 min before proceeding to the next step.
2. Take the unstained samples to the dewaxing robot
3. Log use of dewaxing robot in log sheet
4. Make sure the levels of all reagents in the containers are sufficient. Refresh reagents if longer than 5 days in use and/or if used more than 5 times.
5. Press the button to lift the sample holder arm and load the unstained samples into the slideholder and place the slideholder in the sample holder.
6. Choose program 1.

Program 1:

UltraClear	10min
UltraClear	10min
UltraClear	10min
100% EtOH	5min
100% EtOH	5min
96% EtOH	3min
96% EtOH	3min
90% EtOH	3min
90% EtOH	3min
80% EtOH	3min
70% EtOH	3min
TBS	>5min

7. Start the program.
8. After the program finished, press button to lift arm, transfer slideholder with the slides into a tray containing TBS buffer.

Perform these steps in fume hood.

Epitope retrieval (HIER) and slide staining with antibody mixture

2 1. Prepare decloaking chamber. Add 500 mL of ddH2O to the decloaking chamber, fill all cuvettes that are not used with 200 mL ddH2O and those that will be used with 200 mL of HIER buffer.
2. Select the protocol (30 min, 95 °C) and start the program to heat up the chamber.
3. Once the temperature reaches 95 °C a message will appear on the display prompting the user to load the slides. Open the pressure chamber carefully and place the slideholder with the slides from step 3.3.8 in the tray with HIER buffer.
4. Incubate at 95 °C for exactly 30 min.
5. Open the pressure chamber carefully, place the tray containing the slideholder on bench at room temperature and cool down for 20 min.
6. Transfer the slides into TBS buffer.

3.4 Blocking of unstained samples

3 1. Remove one slide from TBS, tap sideways/gently flick to remove excess buffer, circle each sample on the slide with a hydrophobic barrier pen and place the slide in a wet-chamber.
Caution! If there are multiple samples on the slide make sure that none of them dries out during hydrophobic barrier creation. You may add some TBS on top of the samples to prevent this.
2. Repeat step 1 for all slides.
3. When all tissues have been circled with a pap pen tap sideways or gently flick the slide to remove the TBS and add 100 µL of blocking buffer to each sample. Process one slide after the other.
4. Incubate for 1h at room temperature in wet-chamber.

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It is crucial that during the next procedure the biological material on the slide never dries out.

Antibody Staining

4 1. Prepare the antibody mix. Fill up to the final volume with antibody diluent.
a. If you have a production panel or many antibodies, use the 2x aliquots to prevent a dilution with antibody medium from the antibodies used. Dilute your mix with TBS accordingly so that your mix has the correct final concentration.
2. Add a sufficient volume of the antibody mix to cover the tissue (50 µL/Sample).
3. Close the wet-chamber and move to 4 °C. Ensure tissue is not drying out during incubation by checking that the incubation chamber is completely leveled. Incubate overnight.

DNA Staining and Sample Drying

5 1. Retrieve wet-chamber from 4 °C.
2. Tap sideways/gently flick slides and place in TBS using the tray and the slideholder specific for the slide wash robot.
3. Fill cuvettes from wash station with TBS and start program TBSwash

Program TBSwash

TBS	10 minutes
TBS	10 minutes
TBS	10 minutes

4. During washing, prepare Ir-intercalator solution (50 µL/Sample).
5. Incubate slides with Ir-intercalator solution for 5 min in wet-chamber.
6. Wash slides 3x5 min in TBS as in step 3.
7. Dip slides with stained samples briefly in ddH2O.
8. Dry slides with stained samples under airflow.
9. Store slides with stained samples in a box at room temperature.

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