IMC Staining for Paraffin Sections
(adapted from Fluidigm PN101-5685-A1)

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I. Purpose

This SOP describes the paraffin sections staining procedure for Imaging Mass Cytometry.

II. List of Equipment

1. Slide holder
2. Slide container
3. Fume hood
4. Pressure cooker or heat block (95°C)
5. PAP Pen
6. Centrifuge

III. List of Consumable Reagents

Materials Required	Material Cat No.	Quantity
m-Xylene	185566-1L, Sigma-Aldrich®	50 mL per 5–7 slides
Anhydrous Ethyl Alcohol	USP+432526	172.5 mL per 5–7 slides
Antigen Retrieval Reagent Basic	10mM Tris, 1mM EDTA, pH 9.2	4 mL per 2 slides

10x PBS, pH 7.2	MB-008, Rockland	50 ml per 5-7 slides
Iridium DNA Intercalator	201192B, Fluidigm	2µL per slide
10% Bovine Serum Albumin (BSA)	BSA-50 Rockland Immunochemicals	300µL per slide

IV. Procedure

1. Use pressure cooker or heat block to preheat Antigen Retrieval Solution to 95 °C before starting.
2. Bake slides in 56^{o}C over for 20 min.
3. Dewax slides in xylene in the fume hood for 5 min x2.
4. Hydrate slides in descending grades of ethanol (100%, 95%, 80%, 70%), 5 min each.
5. While you are dewaxing and hydrating slides, prepare 50 mL 1x PBS by diluting 10x PBS with Milli-Q water.
6. Rinse slides in PBS for 5 min.
7. Insert slides with tissues into preheated Retrieval Solution and incubate for 30 min at 95 ^{o}C.
8. Following incubation, remove from pressure cooker or heat block and place the tube containing the retrieval solution and slides on a lab bench and cool to room temperature, approximately 30 min (or until it reaches room temperature).
9. Wash the slide with PBS for 10 min.
10. Use PAP pen to encircle sample.
11. Block with 3% BSA in PBS for 45 min at RT.

NOTE:

- ***Use enough blocking solution to cover the section (around 300–500 µL/section).***
- ***Blocking solution should be diluted from 10% BSA freshly made from powder. Remaining 10% BSA should be aliquoted and stored at −20 °C and diluted at the time of use.***

12. To prepare the antibody cocktail, calculate total volume of antibodies (at concentrations specific for the assay) and bring up to final volume with 0.5%BSA in PBS.

NOTE:

- ***Spin Antibody at max speed for 2 minutes and take from the top of the tube to avoid antibody aggregates.***
- ***Add a small volume of single antibodies into a larger volume of diluent.***

13. Incubate overnight with antibody cocktail at 4˚C in hydration chamber (We use a slide box where the slides rest on the shelf and the bottom is covered by wet paper towel).
14. Wash in PBS for 8 min x 2.
15. Stain the tissue with Ir-Intercalator (1:400) in DPBS for 30 min at RT.
16. Rinse in ddH_2O for 5 min.
17. Air dry the slide for at least 20 minutes at RT.