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Tissue Staining for Imaging Mass Cytometry

John Herndon[1], Madelyn Carmody[1]
[1]Washington University, Saint Louis

1 Works for me This protocol is published without a DOI.

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John Herndon
Washington University, Saint Louis

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ABSTRACT

This protocol describes the preparation and staining of human FFPE material for multiplex visualization using Imaging Mass Cytometry (IMC). This protocol uses basic standard immunohistochemical staining techniques. The tissue is incubated with antibodies that have specific affinity for different cells and tissues in the context of a formalin fixed thin section of human tumor material. Antibodies are labeled with heavy metals instead of the typical fluorochromes, and are visualized using the Hyperion Imaging System manufactured by the Fluidigm corporation.

PROTOCOL CITATION

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GUIDELINES

This technique uses specialized metal tagged antibodies that can be purchased or conjugated by the end user. Dilutions must be empirically determined and validated before using in this protocol. Antibodies are prepared as a cocktail and incubated on the slides all at once.
All containers for reagents, etc. must be in plastic. Glass hardware must not be used, i.e. graduated cylinders, pipettes, bottles, etc. Glass contains lead at low levels and can interfere with heavy metal tagged antibodies.
Slides used must also be kept desiccated and should ideally be freshly cut, do not use any slides that have been cut from blocks for greater than two weeks, if possible.
Oxidation and moisture can interfere with the visualization of the heavy metal tagged antibodies.

MATERIALS TEXT

Xylene, histology grade
Ethanol 100% and diluted in diH20
Tris EDTA pH 9.0 with 10% Glycerol (can be purchased pre made and add glycerol)
Glycerol
PAP pen
Phosphate Buffered Saline (PBS)
Triton X-100
Bovine Serum Albumin (BSA)
DNA intercalator-Ir, [M]**500 Micromolar (µM)** obtain from Fluidigm, catalog # 201192B
Ruthenium Red, Sigma 00541-1G

SAFETY WARNINGS

Xylene and ethanol are volatile chemicals with an inhalation risk. All steps performed with Xylene and Ethanol should be in a certified chemical fume hood.

BEFORE STARTING

Be sure to use clean fresh reagents.

1 2h
Place positively charged slides with **5 µm** thick tissue section in a **60 °C** oven for **02:00:00**

2 20m
Place slides in fresh xylene for **00:20:00**

3 Turn on Water bath for Antigen Retrieval, **96.0 °C**

4 Prepare Antigen retrieval solution of Tris EDTA pH **9.0** with 10% Glycerol

5 Place container for slides with above antigen retrieval solution in to water bath to pre-warm

6 5m
Rehydrate
Place slides in to 100% Ethanol, **00:05:00**

7 5m
Places slides in to 95% Ethanol, **00:05:00**

8 5m
Placed slides in to 80% Ethanol, **00:05:00**

9 Place slides in to 70% Ethanol, **00:05:00** 5m

10 ⚠ ⏸ 5m

Place slides in to distilled water for **00:05:00**

* Note slides may be kept for a longer period at this step, make sure the water bath is the correct temperature before proceeding to the next step.

11 ⚠ 30m

Antigen Retrieval

Incubate slides for **00:30:00** in antigen retrieval solution from step 4

Make sure the **96.0 °C** is maintained consistently throughout the 30 minute incubation.

12 After incubation, cool slides to **70 °C** on the bench top (about 10 minutes)

13 **Wash** 10m

Place slides in distilled water for **00:10:00** with gentle agitation

14 repeat Step 13, **00:10:00** 10m

15 Place slides in PBS for **00:10:00** with gentle agitation 10m

16 repeat Step 15 **00:10:00** 10m

17 **Block Nonspecific Staining**

Encircle each sample with a PAP pen

18 Blocking solution is 3% BSA in PBS, add just enough to cover the sample and incubate for **00:45:00** at **Room temperature** in a hydration chamber. 45m

19 **Antibody Staining**

Pipette **50-100 µl** pre-prepared antibody cocktail in 0.5% BSA and incubate **Overnight** at **4.0 °C** in hydration chamber

20 **Wash** 8m

Wash slides with PBS with 0.2% Triton X-100 for **00:08:00** with gentle agitation

21 Repeat Step 20, **00:08:00** 8m

22 Wash slides in PBS for **00:08:00** with gentle agitation 8m

23 repeat step 22, **00:08:00** 8m

24 **DNA Intercalator-Ir** 30m

Prepare Iridium intercalator by diluting 1 to 1000 in PBS (this dilution may have to determined empirically)

Pipette just enough iridium intercalator to cover sample and incubate for **00:30:00** at **Room temperature**

25 Remove iridium intercalator by gently tapping edge of slide to a paper towel

26 Wash slides with PBS for **00:08:00** with gentle agitation 8m

27 10m

Ruthenium Counter Stain

Prepare a stock solution of Ruthenium Red by mixing 0.0025 grams in PBS, vortex well to mix

Dilute Ruthenium to working concentration of 0.0005%

Counterstain tissue by pipetting just enough of the 0.0005% ruthenium red in PBS for **00:10:00** (duration of ruthenium red may need to be determined empirically)

28 Remove ruthenium red solution by gently tapping edge of slide to paper towel

29 **Wash** 5m

Wash slides with distilled water for **00:05:00** with gentle agitation

30 Air dry slides at room temperature for **00:20:00** 20m

31 Slides are now ready for Hyperion Imaging.