Jul 07, 2022

Tissue staining for IMC

Santhosh Sivajothi[1]

[1]The Jackson Laboratory

1 Works for me Share

This protocol is published without a DOI.

JAX Single Cell Biology
Tech. support email: **scblservice@jax.org**

Santhosh Sivajothi

ABSTRACT

FFPE tissue processing for IMC

PROTOCOL CITATION

Santhosh Sivajothi 2022. Tissue staining for IMC. **protocols.io** https://protocols.io/view/tissue-staining-for-imc-ccwgsxbw

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jul 07, 2022

LAST MODIFIED

Jul 07, 2022

PROTOCOL INTEGER ID

66216

1 Warm slide to 60C for 10 mins on a slide heater

2 Dewax the slide in Histoclear for 20 min in the fume hood.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

3 Rehydrate the slide in descending grades of ethanol (100%, 95%, 90%, 70%, 50%), 5 min each.

4 During rehydration, prepare a coplin jar with 1X antigen retrieval solution and place in pressure cooker to warm.

5 Rinse the slide in distilled, deionized water for 5 min.

6 Insert the slide with tissues into the pre-heated antigen retrieval solution. Perform antigen retrieval under low pressure setting, heating the solution to 95C for 15 mins.

7 Following incubation, release the pressure and place the jar containing the antigen retrieval solution and slides on a lab bench and cool to room temperature, approximately 30 min (or until it reaches room temperature).

8 Rinse the slide with distilled, deionized water for 7 min.

9 Wash the slide with DPBS (no Ca or Mg) for 7 min.

10 Use a PAP pen to encircle the tissue.

11 Block the tissue with 3% BSA in DPBS (no Ca or Mg) or commercial blocking solution such as SuperBlock for 45 min at room temperature. Use enough block solution to cover the tissue, normally around 300 µl/section.

12 Determine antibody working concentrations by prior experiments using a series dilution of identical antibody conjugates.

13 Prepare antibody cocktails. Microcentrifuge metal-conjugated antibodies individually at maximum speed for 3 min prior to making the antibody cocktail, and add only the supernatant.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Calculate the total volume of antibodies needed at concentrations specific for the assay (dilute as necessary using 0.5% BSA in DPBS).

14 Incubate tissue sections with antibody cocktail overnight at 4C in a hydration chamber. Antibody cocktail volume should be sufficient to cover the tissue, about 100 µl.

15 Following overnight incubation, wash the slide twice in 0.1% Triton-X in DPBS (no Ca or Mg), each time for 7 min.

16 Wash the slide twice in DPBS (no Ca or Mg), each time for 7 min.

17 Stain the tissue with intercalator-Ir (1:500 dilution) in DPBS (Ca orMg) for 5 min at room temperature.

18 Rinse the slide in distilled, deionized H2O for 1 min.

19 Air-dry the slide under a gentle flow of pressurized air to dry quickly. After drying completely, proceed with acquisition on Hyperion instrument.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited