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IMC STAINING FOR PARAFFIN SECTIONS

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1 Works for me

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Human Islet Research Network



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ABSTRACT

Imaging mass cytometry (IMC) is a multiplexed imaging method that allows samples to be probed with up to forty antibodies at one time. This enables visualization of multiple cell types simultaneously in the native tissue microenvironment. For example, IMC can be applied to study the interactions between endocrine cells and the immune system in pancreata from donors diagnosed with type 1 diabetes. This protocol describes a technique for IMC staining of paraffin sections with a defined antibody panel to identify immune, islet, endocrine, and stromal cell types of the pancreas.

This protocol was adapted from Fluidigm PN101-5685-A1**Date updated: January 11, 2021**

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EXTERNAL LINK

<https://hgap.pmacs.upenn.edu/explore/workflow/imaging-mass-cytometry?protocol=2>

PROTOCOL CITATION

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KEYWORDS

paraffin sections, Imaging Mass Cytometry, HPAP, HIRN

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MATERIALS TEXT

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

List of Consumable Reagents

- [m-Xylene](#) [Sigma](#)
- 1. [Aldrich Catalog #185566-1L](#)
- [Anhydrous Ethyl Alcohol](#) [Contributed by](#)
- 2. [users Catalog #USP+432526](#)
- [Antigen Retrieval Reagent Basic](#) [Contributed by](#)
- 3. [users Catalog #10mM Tris, 1mM EDTA, pH 9.2](#)
- [10x PBS pH](#)
- 4. [7.2 Rockland Catalog #MB-008](#)
- [Iridium DNA](#)
- 5. [Intercalator Fluidigm Catalog #201192B](#)
- [10% Bovine Serum Albumin](#)
- 6. [\(BSA\) Rockland Catalog #BSA-50 Rockland Immunochemicals](#)

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

Procedure

- 1 Use pressure cooker or heat block to preheat Antigen Retrieval Solution to 95°C before starting.
- 2 Bake slides in 56°C over for 20 min.

Dewax slides in xylene in the fume hood for 5 min X 2.

3

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 °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 mins 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 ddH₂O for 5 min.
- 17 Air dry the slide for at least 20 minutes at RT.

Note:

- A subset of images available on PANC-DB, in the donor specific tabs [here](#) are available for viewing as *ECM*, *Immune*, and *Endocrine* merged overlays (on the donor-specific tabs above, navigate to *Imaging mass cytometry* tab and use *Next/previous donor* buttons to move to other donors).
- These images go through hot-pixel removal before channel merging as part of the data analysis step
- The remainder full set of files available for download are original unaltered files

List of IMC panel

- 18 1. Panels used currently

IMC PANEL USED
HLA-ABC
CD31
C-peptide
Glucagon
PDX-1
CD56
NKX6.1
Somatostatin
CD99
CA2
HLA-DR
Pancreatic polypeptide
Anti-Human CD57 (HCD57)-142Nd— 100 Tests
Anti-Human CD14 (EPR3653)-144Nd —25 µg
Anti-Human Nestin (196908)-146Nd— 100 Tests
Anti-Human Pan- Keratin (C11)- 148Nd—25 µg
Anti-Human/Mouse CD11b (EPR1344)- 149Sm—25 µg
Anti-Human/Mouse CD44 (IM7)-150Nd —100 Tests
Anti-Human CD45 (CD45-2B11)- 152Sm—25 µg
Anti-Human CD45 (D9M8I)-152Sm— 25 µg
Anti-β-actin (2F1-1)- 154Sm—25 µg
Anti-Human FoxP3 (236A/E7)-155Gd— 25 µg
Anti-Human CD4 (EPR6855)-156Gd —25 µg
Anti-Human CD68 (KP1)-159Tb—25 µg
Anti-Human CD20 (H1)-161Dy—25 µg
Anti-Human CD8a (C8/144B)-162Dy— 25 µg
Anti-Human CD8a (D8A8Y)-162Dy— 25 µg
Anti-Human pNFkBp65 [S529] (K10x)-166Er—50 Tests

- [Anti-Human CD57 \(HCD57\)-142Nd— 100 Tests](#)
- [Anti-Human CD14 \(EPR3653\)-144Nd —25 µg](#)
- [Anti-Human Nestin \(196908\)-146Nd— 100 Tests](#)
- [Anti-Human Pan- Keratin \(C11\)- 148Nd—25 µg](#)
- [Anti-Human/Mouse CD11b \(EPR1344\)- 149Sm—25 µg](#)
- [Anti-Human/Mouse CD44 \(IM7\)-150Nd —100 Tests](#)
- [Anti-Human CD45 \(CD45-2B11\)- 152Sm—25 µg](#)
- [Anti-Human CD45 \(D9M8I\)-152Sm— 25 µg](#)
- [Anti-β-actin \(2F1-1\)- 154Sm—25 µg](#)
- [Anti-Human FoxP3 \(236A/E7\)-155Gd— 25 µg](#)
- [Anti-Human CD4 \(EPR6855\)-156Gd —25 µg](#)
- [Anti-Human CD68 \(KP1\)-159Tb—25 µg](#)

- [Anti-Human CD20 \(H1\)-161Dy–25 µg](#)
- [Anti-Human CD8a \(C8/144B\)-162Dy– 25 µg](#)
- [Anti-Human CD8a \(D8A8Y\)-162Dy– 25 µg](#)
- [Anti-Human pNFkBp65 \[S529\] \(K10x\)-166Er–50 Tests](#)

2. Panel combinations/versions used so far

Element	Mass	Panel v1.0 Target	Panel v1.1 Target
In	115		CK19
Pr	141	HLA-ABC	HLA-ABC
Nd	142	CD57	CD57
Nd	143	CD31	CD31
Nd	144	CD14	CD14
Nd	145	C-peptide	C-peptide
Nd	146	Nestin	Nestin
Sm	147	Glucagon	Glucagon
Nd	148	pan-Keratin	pan-Keratin
Sm	149	CD11b	CD163
Nd	150	CD44	CD44
Eu	151	PDX-1	PDX-1
Sm	152	CD45	CD45
Eu	153	CD56	CD56
Sm	154	beta-actin	beta-actin
Gd	155	Foxp3	Foxp3
Gd	156	CD4	CD4
Gd	158	NKX6.1	NKX6.1
Tb	159	CD68	CD68
Gd	160	Somatostatin	Somatostatin
Dy	161	CD20	CD20
Dy	162	CD8	CD8
Dy	163		p16
Dy	164	CD99	CD99
Ho	165	CA2	CA2
Er	166	NFkB	pNFkB
Er	167	Granzyme B	Granzyme B
Er	168	Ki67	Ki67
Tm	169	Collagen type I	Collagen type 1
Er	170	CD3	CD3
Yb	171		IAPP
Yb	172	pS6	CHGA
Yb	173	CD45RO	CD45RO
Yb	174	HLA-DR	HLA-DR
Lu	175	pp	pp
Yb	176	Ghrelin	Ghrelin