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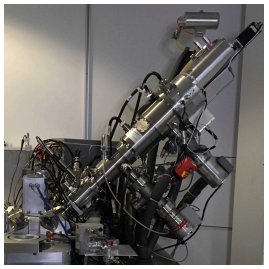
MIBI: MIBI staining of fresh-frozen/OCT-embedded samples



Forked from a private protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol entails the recommended staining procedure for Multiplex Ion Beam Imaging Time of Flight instrument (MIBI_TOF) as developed in the Sean C. Bendall and Michael R. Angelo labs, and has been adapted in the lab of Felix Hartmann specifically for the staining of fresh-frozen samples.

Citation

Marc Bosse, Sean Bendall, Mike Angelo. MIBI staining. protocols.io.

https://protocols.io/view/mibi-staining-b9b3r2qn LINK

Guidelines

Staining tissue sections is fairly straightforward but there are few things to be cognizant of, when preparing samples:

- Always try to limit mechanical damage to the sample surface. This can occur when moving the samples with forceps, particularly when the mounting substrates are small.
- Once the samples have been rehydrated, they cannot dry out until the end of the protocol.
- Be careful at all times to not touch the tissue, in order to not leave any residue.
- Once the samples have been stained, fixed, and dehydrated, they have an indefinite shelf life and can be imaged at any time but need to be stored properly such as in a vacuum chamber or in a sealed vacuum bag
- MIBI_TOF observes the basic principles of Mass spectrometry. Any contaminant ions present in water or in the air can potentially compromise the integrity of the sample.
- Therefore to prevent potential contamination, it is important to **always use single use lab ware containers** to make the solutions. The protocol has been validated using the level of precision of graduated Nalgene bottles and 50 mL tubes.
- **The use of washed beakers or graduated cylinders is not considered as good a practice, due possible introduction of contaminants (exemples of sources: barium from lab ware soap or calcium from air dried lab ware)**

Materials

	A	B	C
	Products	**Provider**	**Catalogue No.**
	Alcohol ethyl ETHANOL 200 PROOF	Gold Shield	412811
	Alcohol ethyl ETHANOL 190 PROOF	Gold Shield	412602
	Target Retrieval Solution, pH 9, (3:1)	Agilent (Dako)	S2375
	UltraPure water	Invitrogen	10977-015
	Gelatin (cold water fish skin)	Sigma-Aldrich	G7765-250
	Glutaraldehyde 8% Aqueous Solution EM Grade	EMS	16020
	Bovine Albumin (BSA), heat shock treated	Fisher	BP1600-100
	Centrifugal filters (0.1µm)	Millipore	UFC30VV00
	MIBI slides	IonPath	567001
	Horse serum	Vector Labs	S-2000
	Equipments	**Provider**	**Cat No.**
	Thermo Scientific™ Lab Vision™ PT Module	Thermo Fisher Scientific	A80400012
	Leica ST4020 Small Linear Stainer	Leica	14050946425
	Bel-Art™ SP Scienceware™ Lab Companion Cabinet Style Vacuum Desiccators, Clear	Fisher Scientific	08-648-109
	Seqenza staining rack + cover plates	Fisher Scientific	73-310-017/72-110-017

Troubleshooting

Safety warnings

All organic solvents should be manipulated under a chemical hood.

Before start

Verify the stocks of all reagents and place an order or prepare solutions, if some reagents are running low.

Initial comments

1 If using frozen and vacuumed slides (e.g. from collaborators), let slides come to room temperature **before destroying the vacuum seal**! (prevent condensation!) ~5-10min

2 In the morning: heat up **PT Module** and prepare fresh antigen retrieval solution (step 6) to place in the device for preheating

Buffer preparation

20m

3 Prepare 1x PBS by diluting 20xPBS 1:20 in Ultrapure type 1 water (needed for blocking buffer)

4 Prepare Blocking buffer (also used as antibody diluent in this protocol!)
This buffer does NOT contain Tween!

	A	B
	Reagents	**Qty for 5mL**
	PBS 1x	4750µL
	Horse Serum	250µL

Constituents of blocking buffer

Filter with .45µm syringe

5 Verify stock of 1x PBS wash buffer and prepare accordingly if running low
This buffer does NOT contain Tween!

	A	B
	Reagents	**Quantity of 250mL**
	PBS 10x (ml)	25mL
	Bovine Albumin (BSA), heat shock treated (g)	0.25g
	Ultrapure (type 1) water (mL)	225mL

Constituents of PBS washing buffer

6 To prepare **fresh antigen retrieval solution**, dilute 10x DAKO (3-in-1) pH9 antigen retrieval solution 1:10 in ultrapure (type 1) water (e.g. for 25mL: 2.5mL + 22.5mL)

note: if retrieving in gold slide delivery chamber (5 slots), 25mL are sufficient

	A	B	C
	Total volume (mL)	Volume target retrieval (mL)	Volume (mL) ddH2O
	25	2.5	22.5
	50	5	45
	100	10	90

Dilution table for Dako Antigen retrieval solution

Thaw and fix, wash

1h

7 If embedding medium is still on slide, carefully dip slide into 1x PBS before fixation

8 Fix Slides after thawing in 10% Neutral Buffered Formaline (NBF) for 1h at room temperature

1h

9 Transfer the slides in the **first** MIBI 1x PBS wash buffer and dip shortly to remove PFA

10 Transfer the slides to the **second** 1x PBS wash buffer and dip shortly to remove PFA

Antigen retrieval after fixation

40m

11 After washing, transfer slide into antigen retrieval buffer jar within the preheated PT module

30m

press run on the digital screen as soon as the device is preheated to 75°C. The display will show **"WARMUP" and heat up to 80°C for retrieval**, stay at this temperature for 20minutes, direcly put tissue back to RT.

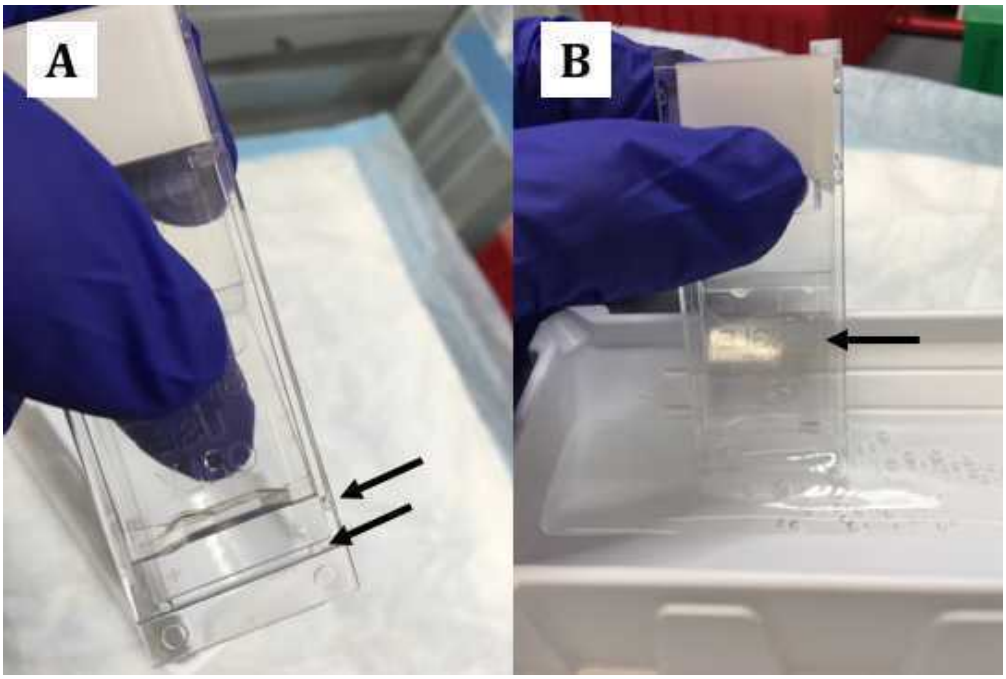
This process takes ~30min.

12 Take slides out of the PT module and let them cool to room temperature (~10 min) before proceeding. 10m

Sequenza assembly

13 Fill a disposable Pipetting Reservoir with 20 mL of 1x PBS wash buffer

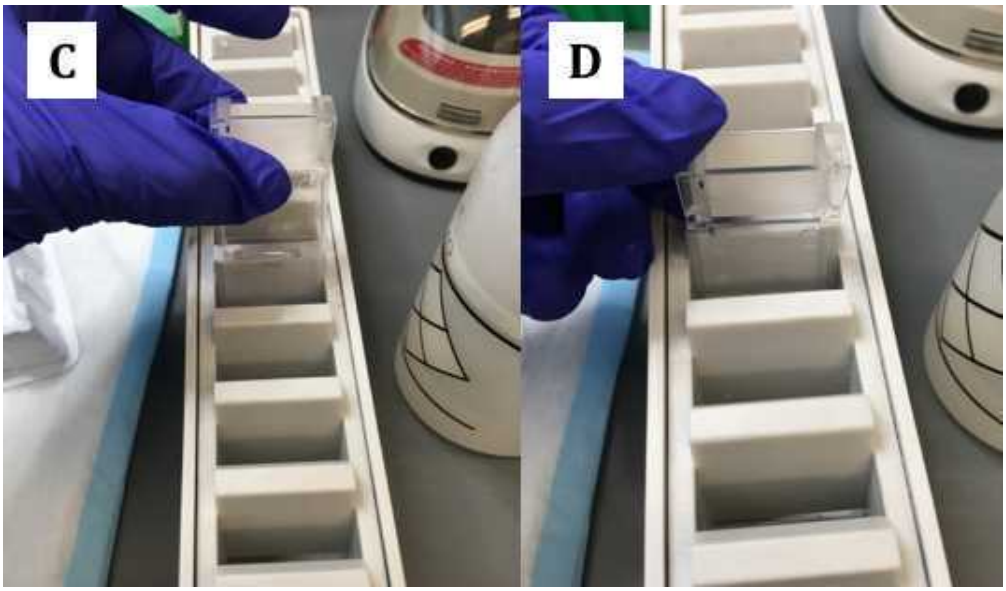
14 Place sample slide on a Sequenza cover plate aligning the bottom slide with the notches on the cover plate (see picture below panel A)



15 Fill by capillarity the space between the slide and cover plate by holding the parts tight and dipping the bottom part of the assembly in the wash buffer reservoir (see picture above panel B).

If capillary force is not sufficient to fill the staining reservoir, a careful pumping motion on the triangular protrusion can help.

16 Transfer the slide and coverplate assembly into the Sequenza rack. Slip in the assembly (see below picture panel C)



17 Secure the assembly and make sure that the assembly placed down in the rack (see above picture panel D)

18 Add 1 mL of wash buffer. The buffer should flow thru within 1 min 30 s. Repeat by adding 1 mL of wash buffer 5m

Blocking 1h

19 Add 200µL of blocking buffer to the sequenza assembly, make sure the buffer is retained in the assembly (the waterline should stop at the upper end of the capillary space)

20 Incubate at Room temperature for 01:00:00 1h

Multiplex Antibody mix

21 Prepare antibody mix based on the putative multiplex antibody panel

Make sure that all the antibodies are ready to use **BEFORE** starting to build the panel

It is highly recommended to prepare all the antibodies, ready to use, a day before the panel is built

21.1 The total volume needed for staining each Sequenza slide assembly is 120 µL

Note

The manufacturer recommend to use 100 µL per Sequenza slide assembly. 120 µL is therefore a 20% excess.

21.2 Build an antibody mix table information to make the antibody panel as follow:

Conjugation ID, Target name, Channel, Antibody concentration, Titer, Volume

Exemple:
The total volume needed for staining each Sequenza slide assembly is 120 µL

	A	B	C	D	E	F
	ID	**Target**	**Channel**	**Concentration µg/mL**	**Titer (µg/mL)**	**Volume (µL)**
	1565	CD45	169	50	0.25	0.75
	1516	CD8	158	50	0.5	1.5
	...	...	...	...	...	...
					Total	150
					Antibody mix	2.25
					Blocking buffer	147.75

For **Blocking Buffer** solution preparation refer to **step 4**

21.3 **Prepare the antibody mix** according to the calculation. Make sure to spin down antibody vials before pipetting to prevent pipetting of aggregates. Use filter tips when pipetting from communal stocks and keep the tubes on ice.

22 **Pre-wet spin column**

Add 400 µL of clean blocking buffer to a Centrifugal 0.1 µm filter unit (Millipore, UFC30VV00)

22.1 10000 rcf, Room temperature, 00:01:00 , discard flowthrough 1m

23 Add antibody mix to the filter unit

23.1 10000 rcf, Room temperature, 00:01:00 1m

Stain 1 (Overnight)

24 Add filtered antibody master mix (120µL) onto the sequenza assembly

25 Place the moist chamber at 4˚C Overnight , preferably in a place with low disturbance 10h

Post staining washing step

26 Following overnight incubation, wash twice with 1 mL of wash buffer

27 After the 1h incubation, wash twice with PBS wash buffer within the sequenza assembly by pipetting 2× 1mL.

Prepare solutions

28 **Prepare fresh 2% glutaraldehyde fixing solution**

Glutaraldehyde fixing solution

1. Add 30 mL of 1x PBS low barium in a 50 mL tube
2. Break the glass glutaraldehyde 8% (amber vial)

3. Add the glutaraldehyde (10 mL) to the diluent by inverting it and tapping the bottom of the vial
4. Transfer the content in a linear stainer container

29 **Set the linear stainer containers**

Fill containers with the following solution and order

Glutaraldehyde x 1, PBS low barium x 1, TRIS 100 mm pH 8.5 × 3, ddH2O x 2, 70% Ethanol x1, 80% Ethanol x1, 95% Ethanol x 2, 100% Ethanol x 2, exit stainless steel tank = empty

Glutaraldehyde fixation

30 Disassemble the sequenza setup. Make sure at this point that the slide doesn't dry out and directly proceed to the next steps.

31 Mount the slides on the linear slide holder

Fix in 2% glutaraldehyde for 00:05:00

32 Rinse briefly with 1x PBS low barium

Dehydration and Storage 2h 10m

33 Press on **Menu** 10m

Check for Processing time = 30 sec, Lift bar = 976, Number of dips = 3

Continue to press **Menu** until the screen displays **Start at: __**

Set Start position corresponding to the first slide carrier position

Exemple: If the first slide carrier is at position 3, use Plus (**+**) or Minus (**-**) button to increase or decrease to get **Start at: 03**

33.1 Then press **Enter**

33.2 Press **Run** on the Linear Stainer

33.3 Allow the dehydration process and wait until the slides reached the **empty stainless steel tank** and stop

33.4 Store the slides immediately under vacuum until MIBI acquisition 2h

Alternatively, the stained slides can be stored in a vacuum sealed bag for longterm storage pre and post MIBI acquisition