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Fixation Protocol for Fresh Frozen Tissue Samples (post-MALDI)

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

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Abstract

Scope:
To provide a method for removal of MALDI matrix, fixation and preparation of tissue sections for Cell DIVE.

Guidelines

Special Notes:
1. Make sure no tissue drying, particularly after matrix removal.
2. Glass petri dishes can be used for more fragile tissues.
3. This protocol can be used on Cell DIVE fresh frozen samples.

Materials

Materials:
1. ITO slides with tissue from MALDI experiment
2. Coplin staining dish, Fisher S17495
3. Glass petri dish, Fisher 08-747C
4. Tabletop shaker

Reagents:
1. 4% paraformaldehyde in PBS, Fisher AAJ61899AK
2. Phosphate Buffered Solution (PBS), Fisher 70-011-044
3. Bovine Serum Albumin, Fisher BP671-10
4. Normal Donkey Serum, Jackson ImmunoResearch 017-000-121
5. Tween 20, Sigma P9416
6. Milliq ddH_2O

Solutions:
1. 1x PBS
   100mL 10x PBS
   900mL ddH_2O

   1000 mL total solution
2. Block Solution:
   2.5 grams of BSA
   5 mL of reconstituted donkey serum (reconstituted in 10mL of ddH_2O)
   0.25 mL of Tween 20
   44.75 mL of 1X PBS
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   50 mL total block solution

Troubleshooting

Safety warnings

Paraformaldehyde should be used inside a chemical fume hood.
For research use only.

1 After the tissue has been imaged via MALDI IMS, the slide should be placed in a coplin jar or glass petri dish with a solution of room temperature 4% paraformaldehyde in PBS (*must be room temperature).

2 Place jar/dish on a shaker for 5 minutes.

3 Discard the PFA (the MALDI IMS matrix will come off the slide in this step).

4 Add fresh 4% PFA in PBS and shake for 5 more minutes (for a total of 10 minutes in PFA).

- Washing with PFA solution will fix and rehydrate the tissue as the matrix is removed. This preserves the tissue and antigens, and significantly reduces artifacts.

5 Add fresh PBS for 3 minutes on shaker.
Repeat this wash *two more times* for a total of 3 times for 3 minutes each.

- pH should be neutral

6 Incubate the slides in 50 mg/mL bovie serum albumin (BSA), 10% (v/v) host-derived serum (depends on which antibodies they use), and 0.5% (v/v) Tween-20 in PBS for 30 minutes.

6.1
- Be very careful. Tissue will be very fragile.
- This permeabilizes the tissue slowly.
- The Tween cannot be substituted for triton X, as triton X is too harsh and will degrade the tissue.
- 30 minutes works for most. Can do up to 2 hours before the tissue gets very damaged/adherence issues/weird background.

7 Add fresh PBS for 3 minutes on shaker.
Repeat this wash *two more times* for a total of 3 times for 3 minutes each.