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CODEX FFPE Staining and Fixation

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wbei[1]

[1]Stanford University

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Joanna Bi
Stanford University

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We use this protocol and it's working

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Abstract

Detailed protocol for preparing, staining, and fixing FFPE slides for use with Akoya flowcells in the Akoya phenocycler (CODEX). Slides are ready to be used with the Akoya phenocycler and the Akoya protocol following this protocol.

Troubleshooting

Staining

4h 44m 30s

1 Bake slides at 70 °C for at least 1 hour in an oven/incubator 1h

> Note
>
> Work on 8 slides at a time

2 Deparaffinize and rehydrate the slides

2.1 Incubate slides for 00:21:00 in xylene in a coplin jar 21m

2.2 Place slides in ST4020 Linear Staining vial and start the staining protocol

2.3 Each step is 3 minutes
Xylene x3 → 100% EtOH x2 → 95% EtOH x2 → 80% EtOH x1 → 70% EtOH x1 → ddH2O x3 36m

> Note
>
> It is okay for some of the slides to only have 2 xylene and/or 2 ddH2O steps. If using the ST4020 Linear Stainer, you can move the slides in the front vial behind the slides in the back vial for the xylene, and remove the slides in the front vial once it has 3 ddH2O steps. Keep slides in ddH2O until the next step

2.4 After starting the linear staining step, fill slide chamber with HIER 1X buffer and incubate HIER buffer at 75 °C (170 °F) in pressure cooker filled with enough water (cover chamber with aluminum foil)

2.5 Transfer slides to chamber containing heated HIER buffer

2.6 Put the chamber back in the pressure cooker, heat to 97 °C (205 °F) and incubate for 00:17:30 min 17m 30s

> Note
>
> Temperature and incubation time is crucial here

2.7 Stop the pressure cooker and turn it off, leave the chamber in the water bath for 00:20:00 to cool down slowly. 20m

2.8 Take the chamber out of pressure cooker, cool down at RT for about 00:30:00 until around RT 30m

3 Wash tissue

3.1 Place slide in coplin jar containing 80 mL of 1X TBS IHC Wash Buffer with Tween 20 (https://www.cellmarque.com/ancillaries/CM/2087/TBS-IHC-Wash-Buffer-Tween-20)

3.2 Incubate on a shaker for 00:10:00 at around 100 rpm 10m

4 Make blocking buffer solution (amount depends on sample area. ~120-140 uL/slide)
1 mL = 780 uL of S2 (RT), 50 uL of B1, 50 uL of B2, 50 uL of B3, 70 uL of BC4
NOTE: can store remaining buffer at 4°C

5 Block

5.1 Tap off excess wash buffer, wipe edges and back with Kim Wipes, and place slides in humidity chamber (or use pipette box with water and paper towel underneath)

5.2 Add 120-140 ul (depending on the sample area) of blocking buffer and incubate for 01:00:00 at RT in humidity chamber 1h
(Can leave much longer and just need to add solution so tissue does not dry out)

6 Dilute antibodies in blocking buffer to a total of 120 ul (ratio of blocking buffer: antibody cocktails should be >= 1 v/v).

7 Antibody Staining

7.1 After 1 hour of blocking, tap off excess buffer and add 120 ul of conjugated antibody solution. Cover tissue area with Parafilm

7.2 Incubate Overnight at 4 °C in humidity chamber on a shaker 30m

Fixation 42m

8 *Wash tissue*.

8.1 Place slide in chamber containing S2 buffer.

8.2 Incubate for 00:04:00 on a shaker 4m

9 *Fix tissue*. Prepare 1.6% PFA (dilute from 16% PFA) solution in S4 buffer (1:10 (v/v)).
NOTE: Use fresh vial of PFA every 1-2 weeks.

9.1 Place slide in humidity chamber and add 100 uL of PFA solution or enough to cover the tissue

9.2 Incubate for 00:10:00 . 10m

10 *Wash tissue.*

10.1 Place slide in chamber containing 1x PBS for 00:01:00 on a shaker 1m

11 *Ice-cold methanol incubation*. Place slide chamber on an ice and fill with cold methanol (4 °C).

11.1 Remove slide from chamber containing 1x PBS and place in chamber containing ice-cold methanol.

11.2 Incubate for 00:05:00 . 5m

12 *Wash tissue*.

12.1 Remove slide from cold methanol and place in chamber containing 1x PBS (ok to use same PBS as from step #10).

12.2 Incubate for 00:01:00 on a shaker 1m

13 *Fix tissue*. Prepare final fixative solution. Remove FIX aliquot from -20°C freezer right before use and let it melt. Add entire contents (~20 ul) to 1 ml of 1x PBS. Mix fully.

13.1 Add 100 uL of fixative solution (or enough to cover the tissue), taking care not to pipette directly onto tissue.

13.2 Incubate for 00:20:00 in a humidity chamber. 20m

14 *Wash tissue*.

14.1 Place slide in chamber containing 1x PBS for 00:01:00 on a shaker 1m

15 Assemble the Akoya flowcells to the slides directly or store slides in S4 buffer, and assemble later.