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CODEX Staining Protocol For FFPE Tissue

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

CHOP TMC CODEX on FFPE Tissue

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This protocol describes the method for antibody staining of FFPE tissues on coverslips using CODEX Barcoded Antibodies. The Akoya CODEX user manual was modified to include photobleaching steps. Included are the stepwise protocols for pre-staining, deparaffinization, antigen retrieval, photobleaching, antibody staining and post-fixation.

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- Use poly-l-Lysine coated coverslips.
- Keep tissue sections from drying during transfer steps

CODEX® Staining Kit (Akoya PN: 7000008)

Tissue Pre-treatment 25m

1 Heat sample coverslip on hot plate at 55°C with tissue facing up for 20-25 minutes until wax thoroughly melts. 20m

2 Place the sample coverslip in the coverslip staining rack and wait 5 minutes to allow the tissue to cool down. 5m

Tissue Deparaffinization and Hydration 50m

3 Immerse the staining rack in the container containing the following reagents for 5 minutes each: 50m

a.Xylene
b.Xylene
c.100% Ethanol/Reagent Alcohol
d.100% Ethanol/Reagent Alcohol
e.90% Ethanol/Reagent Alcohol
f.70% Ethanol/Reagent Alcohol
g.50% Ethanol/Reagent Alcohol
h.30% Ethanol/Reagent Alcohol
i.ddH2O
j.ddH2O

Antigen Retrieval 45m

4 In a 50 ml Pyrex beaker, prepare 40 mls of a 1x citrate buffer (0.01 M). Dilute 100x citrate buffer pH6.0 to 1X citrate buffer in ddH2O.

5 Immerse the staining rack in the beaker containing the 1x Citrate buffer and wrap it with aluminum foil to ensure the best sealing possible.

6 Fill the pressure cooker with water approximately halfway up the height of the beaker. Place the aluminum foil wrapped beaker in the pressure cooker.

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7 Set the pressure cooker to the high-pressure protocol and let the tissue incubate for 20 min. 30m

8 After incubation in the pressure cooker, release the pressure and carefully remove the rack from the pressure cooker and allow to cool on the bench for 15 minutes until no longer hot to the touch. 15m

Wash Tissue 6m

9 Place staining rack in a beaker containing 40 ml of ddH2O for 2 minutes. 2m

10 Place the staining rack in a second beaker filled with ddH2O and incubate for 2 minutes. 2m

11 Place the staining rack in a third beaker filled with 1X PBS and incubate for 2 minutes. 2m

Photobleach Tissue 1h 30m

12 Submerge the sample coverslip in a well of a 6-well plate containing 5 mls of 4.5% (w/v) H2O2 and 20mM NaOH in PBS (bleaching solution).

13 Sandwich the 6-well plate between two broad-spectrum LED light sources for 45 minutes at 4°C. 45m

14 After 45 minutes move the sample coverslip(s) into a new well with fresh bleaching solution and photobleach for another 45 minutes at 4°C. 45m

Wash Tissue 10m

15 Wash the sample coverslip three times in 1x PBS for 2 minutes. 6m

16 Immerse the sample coverslip in a well of a 6-well plate containing 5 mls CODEX® Hydration Buffer and incubate for 2 minutes. 2m

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17 Move sample coverslip to a second well containing CODEX® Hydration Buffer and incubate for another 2 minutes. 2m

Equilibrate Tissue in Staining Buffer 20m

18 Move sample coverslip to a well containing CODEX® Staining Buffer and incubate for 20-30 minutes. 20m

Preparation of the Antibody Cocktail Solution

19 Prepare a stock solution of CODEX® Blocking Buffer to be used for the Antibody Cocktail.

CODEX® Blocking Buffer	1 Sample
Staining Buffer [µL]	181
N Blocker [µL]	4.75
G Blocker [µL]	4.75
J Blocker [µL]	4.75
S Blocker [µL]	4.75
Total [µL]	200

20 Add CODEX® Blocking Buffer to a tube designated for Antibody Cocktail Staining Solution. The volume of CODEX® Blocking Buffer to be prepared for each sample coverslip can vary depending on the titer and corresponding volume of each antibody. Adjust volume of CODEX® Blocking Buffer so that the final volume of the Antibody Cocktail Staining Solution is a total of 200 µL per tissue.

21 Add the appropriate volume of each CODEX Antibody to the Antibody Cocktail Solution.

22 Pipette to mix and briefly spin down the tube.

Tissue Staining 16h

23 Remove sample coverslip from the well containing Staining Buffer and place it on the tray of a humidity chamber.

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24 Add 190 µL of the Antibody Cocktail to the top corner of the sample coverslip and ensure that the liquid covers the entire tissue.

25 Incubate overnight in the humidity chamber at 4°C. 16h

Wash Tissue 4m

26 After overnight antibody incubation, immerse the sample coverslip in a well of a 6-well plate containing 5 mls CODEX® Staining Buffer for 2 minutes. 2m

27 Place sample coverslip in a second well containing CODEX® Staining Buffer for 2 minutes. 2m

Fix Tissue 10m

28 Place sample coverslip in a well containing 5 mls of 1.6% PFA in CODEX® Storage Buffer and incubate for 10 minutes. 10m

Wash Tissue 1m

29 Transfer coverslip to a well of a 6-well plate containing 5 mls of 1X PBS and immerse sample coverslip 2-3 times.

30 Transfer coverslip to a second well of 1X PBS and immerse sample coverslip 2-3 times.

31 Transfer coverslip to a third well of 1X PBS and immerse sample coverslip 2-3 times.

Ice-cold Methanol Incubation 5m

32 Transfer coverslip to well of another 6-well plate containing Ice cold methanol and incubate for 5 minutes on ice. 5m

Wash Tissue 1m

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33 Place the 6-well plate containing the previously used 1x PBS next to the ice bucket and the methanol tray containing the sample coverslip. Quickly transfer the sample coverslip from methanol to the first corresponding 1x PBS well and immerse sample coverslip 2-3 times.

34 Transfer the sample coverslip to the second 1x PBS well and immerse sample coverslip 2-3 times.

35 Transfer the sample coverslip to the third 1x PBS well and immerse sample coverslip 2-3 times.

Fix Tissue 20m

36 Prepare the Final Fixative Solution by diluting the 20 µL of the CODEX® Fixative Reagent in 1 mL of 1x PBS.

37 Remove sample coverslip from the well and place it on the tray of a humidity chamber.

38 Add 200 µL of Final Fixative Solution to the top corner of the sample coverslip and ensure that the entire tissue is covered in fixative solution.

39 Incubate for 20 mins. 20m

Wash Tissue 1m

40 Remove the sample coverslip from the humidity chamber and place in the first well containing previously used 1x PBS. Lift and immerse the sample coverslip 2-3 times.

41 Move the sample coverslip to the second well containing 1x PBS and immerse sample coverslip 2-3 times.

42 Move the sample coverslip to the third well containing 1x PBS and immerse sample coverslip 2-3 times.

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Store Tissue

43 Transfer sample coverslip to a well of a 6-well plate containing 5 mls of CODEX® Storage Buffer.

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