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Phenocycler-Fusion 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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ABSTRACT

This protocol describes the method for antibody staining of FFPE tissues on slides using CODEX barcoded antibodies. The Akoya Phenocycler-Fusion 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.

GUIDELINES

- Use positively charged slides.
- Keep tissue sections from drying during transfer steps

MATERIALS

Sample Kit for PhenoCycler-Fusiont (Akoya PN: 7000017)

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

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Tissue Pre-treatment

1h 5m

1 Bake sample slide/s in an incubator at 65°C for 1-3 hours until paraffin thoroughly melts. 1h

2 Cool sample slide/s at room temperature for 5 minutes to allow the sample slide/s to cool to room temperature. 5m

Tissue Deparaffinization and Hydration

50m

3 Immerse sample slide/s in a coplin jar containing the following reagents for 5 minutes each: 50m

a.Histochoice
b.Histochoice
c.100% Ethanol
d.100% Ethanol
e.90% Ethanol
f.70% Ethanol
g.50% Ethanol
h.30% Ethanol
i.ddH2O
j.ddH2O

Antigen Retrieval

45m

4 In a coplin jar, prepare 50 mls of a 1x citrate buffer. Dilute 10x citrate buffer pH6.0 to 1X citrate buffer in ddH2O.

5 Immerse sample slide/s in the coplin jar containing the 1x Citrate buffer and cover the coplin jar with aluminum foil.

6 Fill the pressure cooker with 1200 mls of ddH2O. Place the aluminum foil covered coplin jar containing sample slide/s in the pressure cooker.

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

8 After incubation in the pressure cooker, release the pressure and carefully remove the coplin jar from the pressure cooker and allow to cool at room temperature for 30 minutes. 15m

Wash Tissue

6m

9 Place sample slide/s in a coplin jar containing 50 mls of ddH2O for 2 minutes. All volumes for coplin jar incubations will be 50 mls henceforth in this protocol. 2m

10 Place sample slide/s in a second coplin jar filled with ddH2O and incubate for 2 minutes. 2m

11 Place sample slide/s in a third coplin jar filled with 1X PBS and incubate for 2 minutes. 2m

Photobleach Tissue

1h 30m

12 Submerge the sample slide/s in a 150 mm petri dish containing 50 mls of 4.5% (w/v) H2O2 and 20mM NaOH in PBS (bleaching solution).

13 Sandwich the petri dish 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 petri dish with fresh bleaching solution and photobleach for another 45 minutes at 4°C. 45m

Wash Tissue

10m

15 Wash the sample slide/s three times for 2 minutes each in coplin jars containing 1X PBS. 6m

16 Immerse the sample slide/s in a coplin jar containing 50 mls of CODEX® Hydration Buffer and incubate for 2m minutes.

17 Move sample slide/s to a second coplin jar containing CODEX® Hydration Buffer and incubate for another 2m minutes.

Equilibrate Tissue in Staining Buffer

20m

18 Move sample sample slide/s to a coplin jar containing 50 mls of CODEX® Staining Buffer and incubate fo 20m 20-30 minutes.

Preparation of the Antibody Cocktail Solution

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

	A	B
	CODEX® Blocking Buffer	1 Sample
	Staining Buffer [µL]	181
	N Blocker [µL]	4.75
	G2 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 slide/s 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 slide/s from the coplin jar containing Staining Buffer and place it on the tray of a humidity chamber.

24 Add 190 µL of the Antibody Cocktail to each of the sample slide(s) 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 slide/s in a coplin jar containing 50 mls of CODEX® Staining Buffer for 2 minutes. 2m

27 Place sample slide/s in a second coplin jar containing CODEX® Staining Buffer for 2 minutes. 2m

Fix Tissue 10m

28 Place sample slide/s in a coplin jar containing 50 mls of 1.6% PFA in CODEX® Storage Buffer and incubat 10m for 10 minutes.

Wash Tissue 1m

29 Transfer sample slide/s to a coplin jar containing 50 mls of 1X PBS. Lift and immerse the sample slide/s 2-3 times.

30 Transfer sample slide/s to a second coplin jar of 1X PBS and immerse sample slide/s 2-3 times.

31 Transfer sample slide/s to a third coplin jar of 1X PBS and immerse sample slide/s 2-3 times.

Ice-cold Methanol Incubation

5m

32 Transfer sample slide/s to a coplin jar containing Ice cold methanol and incubate for 5 minutes on ice. 5m

Wash Tissue

1m

33 Place a coplin jar of 1x PBS next to the methanol coplin jar containing the sample slide/s. Quickly transfer the sample slide/s from methanol to the coplin jar of 1x PBS and immerse sample slide/s 2-3 times.

34 Transfer the sample slide/s to a second coplin jar of 1x PBS and immerse sample slide/s 2-3 times.

35 Transfer the sample coverslip to a third coplin jar of 1x PBS and immerse sample slide/s 2-3 times.

Fix Tissue

20m

36 Prepare the Final Fixative Solution by diluting 20 µl of the CODEX® Fixative Reagent in 1 ml of 1x PBS (Final Fixative Solution).

37 Remove sample slide/s from the coplin jar and place it on the tray of a humidity chamber.

38 Add 200 µL of Final Fixative Solution to each of the sample slide/s and ensure that the entire tissue is covered in fixative solution.

39 Incubate for 20 mins. 20m

Wash Tissue

1m

40 Remove the sample slide/s from the humidity chamber and place in a coplin jar containing 1x PBS. Lift and immerse the sample slide/s 2-3 times.

41 Move the sample slide/s to a second coplin jar containing 1x PBS and immerse sample slide/s 2-3 times.

42 Move the sample slide/s to a third coplin jar containing 1x PBS and immerse sample slide/s 2-3 times.

Store Tissue

43 Transfer sample slide/s to a coplin jar containing 50 mls of CODEX® Storage Buffer and store at 4°C until imaging.