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## Post-PhenoCycler Fusion Manual Hematoxylin and Eosin (H&E) Staining of Female Reproductive System Tissues

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**We use this protocol and it's working**

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## Abstract

This protocol describes a standardized method for staining formalin-fixed paraffin-embedded (FFPE) tissue sections with hematoxylin and eosin (H&E) after Akoya Phenocycler-Fusion imaging. The procedure allows for removal of the Akoya flowcell and does not require it to remain mounted on the slide. H&E staining following multiplex imaging enables histopathological inspection of tissue morphology and integrity.



## Guidelines

- Use appropriate handling and personal protective equipment when handling any human samples.
- Use appropriate handling and personal protective equipment when any flammable substance :

⌘ Xylenes (ACS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #247642-2.5L**

⌘ Ethanol **Decon Labs Catalog #2716**

## Materials

### Acid Alcohol:

-  50 µL Hydrochloric acid (HCl)
-  25 mL Ethanol
-  24.950 mL dH<sub>2</sub>O

 Hydrochloric Acid, Certified ACS Plus, 36.5 to 38.0%, Fisher Chemical™ **Fisher Scientific Catalog # A144-500**

### 95% Ethanol

-  95 mL Ethanol
-  5 mL dH<sub>2</sub>O

 Ethanol **Decon Labs Catalog #2716**

### 80% Ethanol

-  80 mL Ethanol
-  20 mL dH<sub>2</sub>O

### 70% Ethanol

-  70 mL Ethanol
-  30 mL dH<sub>2</sub>O

## Equipment

3DHistech Pannoramic MIDI II Digital Scanner

NAME

Digital Slide Scanner

TYPE

3DHistech

BRAND

whole-slide digital slide scanner

SKU

[https://www.fishersci.com/shop/products/3dhistech-pannoramic-midi-ii-digital-scanner-brightfield-60x-resolution-fluorescence-60x-resolution-sola/M2BFFLSOLS#?](https://www.fishersci.com/shop/products/3dhistech-pannoramic-midi-ii-digital-scanner-brightfield-60x-resolution-fluorescence-60x-resolution-sola/M2BFFLSOLS#?keyword=DHistech%20Pannoramic%20MIDI%20II%20Digital%20Scanner)

[keyword=DHistech%20Pannoramic%20MIDI%20II%20Digital%20Scanner](https://www.fishersci.com/shop/products/3dhistech-pannoramic-midi-ii-digital-scanner-brightfield-60x-resolution-fluorescence-60x-resolution-sola/M2BFFLSOLS#?keyword=DHistech%20Pannoramic%20MIDI%20II%20Digital%20Scanner)

LINK

 3DHistech Pannoramic MIDI II Digital Scanner

## Protocol materials

⊗ Xylenes (ACS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #247642-2.5L**

⊗ Mayer's Hematoxylin **Dako Catalog #S3309**

⊗ Eosin Y solution aqueous **Merck Catalog #HT110216-500ML**

⊗ Permount mounting medium **Fisher Scientific**

⊗ Ethanol **Decon Labs Catalog #2716**

⊗ Hydrochloric Acid, Certified ACS Plus, 36.5 to 38.0%, Fisher Chemical™ **Fisher Scientific Catalog # A144-500**

## Safety warnings

- ⚠ Make sure you fully complete the Akoya's PhenoCycler Fusion's protocol and imaging prior to separating the flowcell. H&E staining of the tissue will render the sample unsuitable for processing PhenoCycler technology.

### Protocol

NAME

**Akoya Biosciences PhenoCycler Fusion (formerly CODEX) User Guide**

CREATED BY

Andrew Houston

Preview

## Ethics statement

Research involving humans and animals must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

The sample collection for The Human Biomolecular Atlas Program (HuBMAP): Multi-scale Molecular Mapping of the Female Reproductive System were approved by the University of Pennsylvania Institutional Review Board IRB00000039 (IRB Protocol #844297), and written informed consent was obtained prior to participation.



## Removing Phenocycler-Fusion flowcell

12h

- 1 Post-imaging in the Phenocycler Fusion system, store slide Overnight in Xylenes (ACS) Merck MilliporeSigma (Sigma-Aldrich) Catalog #247642-2.5L at Room temperature to dissolve flowcell adhesion strip.

12h

## H&E Staining

37m 30s

- 2 Rehydration

- 2.1 Soak in Xylenes (ACS) Merck MilliporeSigma (Sigma-Aldrich) Catalog #247642-2.5L for 00:05:00 in a slide holder at Room temperature , repeat soak for a total of two soaks.

10m

- 2.2 Soak in 100% Ethanol for 00:02:00 in a slide holder at Room temperature , repeat twice for a soak for a total of three times.

6m

- 2.3 Soak in 95% Ethanol for 00:02:00 in a slide holder at Room temperature .

2m

- 2.4 Soak in 80% Ethanol for 00:02:00 in a slide holder at Room temperature .

2m

- 2.5 Soak in 70% Ethanol for 00:02:00 in a slide holder at Room temperature .

2m

- 2.6 Soak in deionized water (dH<sub>2</sub>O) in a slide holder twice at Room temperature .

1m

- 3 Staining



- 3.1 Place slide on stain box, pipette between 200  $\mu\text{L}$  to 400  $\mu\text{L}$  of Mayer's Hematoxylin **Dako Catalog #S3309** on top of the slide and incubate for 00:02:30 at Room temperature (this step may be optimized for 1 to 5 minutes) 3m
- 3.2 Wash excess Hematoxylin by dipping the slide in  $\text{dH}_2\text{O}$  in a slide holder a total of three times at Room temperature . Use fresh  $\text{dH}_2\text{O}$  for each dip. 1m 30s
- 3.3 12 dips in Acid alcohol at Room temperature . 30s
- 3.4 Rinse under Room temperature tap water for 00:02:00 and 3 dips in  $\text{dH}_2\text{O}$  2m 30s
- 3.5 Place back slide on stain box, pipette between 200  $\mu\text{L}$  to 400  $\mu\text{L}$  of Eosin Y solution aqueous **Merck Catalog #HT110216-500ML** and incubate for 00:01:00 at Room temperature . 1m
- 3.6 Dip once in  $\text{dH}_2\text{O}$  at Room temperature to wash off excess Eosin Y and immediately proceed to dehydration.
- 4 Dehydration and mounting
- 4.1 Soak in 95% Ethanol for 00:02:00 in a slide holder at Room temperature . 2m
- 4.2 Soak in 100% Ethanol for 00:02:00 in a slide holder at Room temperature . 2m
- 4.3 Soak in Xylenes (ACS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #247642-2.5L** for 00:02:00 in a slide holder at Room temperature . 2m
- 4.4 Air dry completely and mount with 60  $\mu\text{L}$  to 100  $\mu\text{L}$  of Permunt mounting medium **Fisher Scientific** and place the coverslip by angling



the coverslip and let fall gently onto the slide. Allow the Permout to spread beneath the coverslip, covering all the tissue. Seal slide with clear nail polish and image

## Protocol references

### Citation

Sarah Black, Darci Phillips, John W. Hickey, Julia Kennedy-Darling, Vishal G. Venkataaraaman, Nikolay Samusik, Yury Goltsev, Christian M. Schürch, Garry P. Nolan  
(2021). CODEX multiplexed tissue imaging with DNA-conjugated antibodies. Nature Protocols.

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LINK

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