

Jan 26, 2026

Version 1

HEdeX protocol V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvwnz9wvmk/v1>

Fan Wu¹

¹Johns Hopkins University

Fan Wu



wu.

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.e6nvwnz9wvmk/v1>

Protocol Citation: Fan Wu 2026. HEdeX protocol. protocols.io <https://dx.doi.org/10.17504/protocols.io.e6nvwnz9wvmk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: January 24, 2026

Last Modified: January 26, 2026

Protocol Integer ID: 240838

Keywords: hedex protocol, protocol

Abstract

For destaining H&E and restaining



Decoverslipping

1d

- 1 Soak the H&E stained slides in 100% acetone

1d

Rehydration (Eosin destaining)

45m

- 2 Soak the H&E slides without coverslip in 100% Xylene
- 3 Soak the H&E slides without coverslip in 100% EtOH
- 4 Soak the H&E slides without coverslip in 90% EtOH
- 5 Soak the H&E slides without coverslip in 80% EtOH
- 6 Soak the H&E slides without coverslip in 75% EtOH
- 7 Soak the H&E slides without coverslip in 100% H₂O
- 8 PBS wash for 3 times, 5 minutes each

5m

5m

5m

5m

5m

5m

15m

Destaining (Hematoxylin destaining)

55m

- 9 Prepare the destaining solution and mildly soak the slides into the destaining solution (70ml of PBS + 30 ml of 100% EtOH + 500ul of concentrated 37% HCl).
- 10 PBS wash for 3 times, 5 minutes each

40m

15m

Antigen retrieval

35m



11 Add 300 ml of unmasking solution (Antigen unmasking solution, citric acid based, vector, H-3300) into 32 ml of H₂O in a small plate (heat resistant plate, maybe the cap of the antibody box), make sure the water covers the slides.

20m

12 TBST wash for 3 times, 5 minutes each

15m

iCLAP bleaching

1h 15m

13 Tissue sections were placed in a transparent box, which was then filled with the bleaching solution containing 2M H₂O₂ and 3mM EDTA in PBS at pH 12.5. The transparent box, holding the tissue slides and bleaching solution, is positioned between two 5000 lux light pads (HSK, 615517997868) for one hour to facilitate fluorophore inactivation.

1h

14 TBST wash for 3 times, 5 minutes each

15m

Blocking

16h

15 Tissue sections were blocked with BlockerTM casein (Thermo Scientific, 37528) in 4 C fridge overnight

16h

Staining

16 Normal IF, TSA, CODEX or IMC could be applied to the destaining slides after the blocking