Feb 23, 2026 Version 2

HEdeX protocol V.2

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwnz9wvmk/v2

Fan Wu[1]

[1]Johns Hopkins University

Fan Wu

wu.

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwnz9wvmk/v2

Protocol Citation: Fan Wu 2026. HEdeX protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwnz9wvmk/v2Version created by **wu.**

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

Created: February 20, 2026

Last Modified: February 23, 2026

Protocol Integer ID: 243763

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 — 5m

3 Soak the H&E slides without coverslip in 100% EtOH — 5m

4 Soak the H&E slides without coverslip in 90% EtOH — 5m

5 Soak the H&E slides without coverslip in 80% EtOH — 5m

6 Soak the H&E slides without coverslip in 75% EtOH — 5m

7 Soak the H&E slides without coverslip in 100% H2O — 5m

8 PBS wash for 3 times, 5 minutes each — 15m

Destaining (Hematoxylin destaining)

35m

9 Prepare the destaining solution and mildly soak the slides into the destaining solution (70ml of PBS + 30 ml of H2O + 500ul of concentrated 37% HCl).

10 Dip the slide in destaining solution for several times and let it set for 5 minutes. Repeat this step for 3 times. — 15m

11 Wash the slide in EDTA buffer solution (5 mM) for 5 minutes. — 5m

12 PBS wash for 3 times, 5 minutes each 15m

Antigen retrieval 35m

13 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

14 TBST wash for 3 times, 5 minutes each 15m

iCLAP bleaching 1h 15m

15 Tissue sections were placed in a transparent box, which was then filled with the bleaching solution containing 2M H_2O_2 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

16 TBST wash for 3 times, 5 minutes each 15m

Blocking 16h

17 Tissue sections were blocked with Blocker™ casein (Thermo Scientific, 37528) in 4 C fridge overnight 16h

Staining

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