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Manual H&E Staining

Jia Ren
Lin¹

¹Harvard Medical School, Laboratory of Systems Pharmacology

Laboratory of Systems Pharmacology

NCIHTAN

Madison Tyler
Laboratory of Systems Pharmacology

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We use this protocol and it's working

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ABSTRACT

The hematoxylin and eosin (H&E) stain is the standard used for microscopic examination of fixed tissue specimens. This procedure establishes a consistent process for the manual preparation of H&E-stained slides from FFPE tissue specimens. H&E staining is compatible with, and frequently performed after tissue-based cyclic immunofluorescence (t-CyCIF), enabling conventional histopathology review to be combined with fluorescence imaging.

MATERIALS

Required Materials

- Hematoxylin Solution, Gill No. 3 (Millipore Sigma, GHS332)
- Eosin Y Solution, 1% aqueous (Millipore Sigma, HT110232)
- Coverslips, rectangular 50x24 mm (VWR, 48393-081)
- Permount Mounting Medium (Fisher Chemical, SP15-100)
- Staining jar

You may also need:

- Forceps
- 20X Phosphate Buffered Saline (Thermo Fisher Scientific, 28348)

BEFORE START INSTRUCTIONS

- This protocol can be performed on the same tissue specimen that has previously undergone t-CyCIF. A protocol for t-CyCIF can be found here: dx.doi.org/10.17504/protocols.io.bjiukkew.

Post-CyCIF Decoverslipping

30m

1 After the final imaging step of CyCIF (or IF), soak slides in 1X PBS for 00:30:00 Room temperature and gently detach coverslips from slides using slide forceps. 30m

2 Wash slides 1-2 times in the staining jar with tap water Room temperature .

Manual H&E Staining

1m

3 Remove slides and place on the stain box. Add between 200 μ L and 400 μ L Hematoxylin solution and let incubate for 00:01:00 Room temperature . 1m

4 Wash slides by placing them into the staining jar with running tap water for 00:00:30 Room temperature . 30s

5 Put washed slides back to the staining box. Add between 200 μ L and 400 μ L Eosin Y solution and incubate for 00:01:00 Room temperature . 1m

6 Wash slides by placing into the staining jar with running tap water for at least 00:00:30 Room temperature until all the excess red Eosin color is gone. 30s

7 Air dry slides completely (at least 01:00:00), mount slides with 80 μ L Permount solution, and gently place coverslips. Slides can be imaged after Overnight . 1h