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# Manual H&E Staining

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Works for me

[dx.doi.org/10.17504/protocols.io.bsi8nchw](https://dx.doi.org/10.17504/protocols.io.bsi8nchw)

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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.

## DOI

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## PROTOCOL CITATION

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## KEYWORDS

histology, H&amp;E, CyCIF

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## MATERIALS TEXT

### 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 STARTING

- 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](https://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 µl and 🧴 400 µl 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 µl and 🧴 400 µl 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 µl Permount solution, and gently place coverslips. Slides can be imaged after ⌚ Overnight . 1h