



Jan 14, 2025

Yale University Hematoxylin and Eosin (H&E) Staining for Formalin-Fixed Paraffin-Embedded Human Lymph Node Tissues Protocol Adapted from VHIR-CIBERNED-ASAP



Forked from [Hematoxylin and Eosin \(H&E\) Staining](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvo98x9v4o/v1>

Negin Farzad¹, Archibald Enniful¹, Rong Fan¹

¹Yale University

Cellular Senescence Net...



Negin Farzad

Yale University

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.5qpvo98x9v4o/v1>

Protocol Citation: Negin Farzad, Archibald Enniful, Rong Fan 2025. Yale University Hematoxylin and Eosin (H&E) Staining for Formalin-Fixed Paraffin-Embedded Human Lymph Node Tissues Protocol Adapted from VHIR-CIBERNED-ASAP. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5qpvo98x9v4o/v1>

Manuscript citation:

Bai Z, Zhang D, Gao Y, Tao B, Zhang D, Bao S, Enniful A, Wang Y, Li H, Su G, Tian X, Zhang N, Xiao Y, Liu Y, Gerstein M, Li M, Xing Y, Lu J, Xu ML, Fan R. Spatially exploring RNA biology in archival formalin-fixed paraffin-embedded tissues. *Cell*. 2024 Nov 14;187(23):6760-6779.e24. doi: 10.1016/j.cell.2024.09.001. Epub 2024 Sep 30. PMID: 39353436; PMCID: PMC11568911.

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 11, 2025

Last Modified: January 14, 2025

Protocol Integer ID: 118088

Keywords: embedded human lymph node tissue, human lymph node tissues protocol adapted, human lymph node tissue, embedded human lymph node tissues protocol adapted, asap hematoxylin, yale university hematoxylin, eosin, tissue, human lymph node tissues protocol adapted from vhir

Funders Acknowledgements:

Yale TMC for Cellular Senescence in Lymphoid Organs

Grant ID: 1U54AG076043-01

Abstract

Hematoxylin and Eosin (H&E) Staining for for Formalin-Fixed Paraffin-Embedded Human Lymph Node Tissues after sectioning.



DEPARAFFINIZE

- 1 Bake the tissue section on the slide at 60 °C - 1 hr
- 2 Immerse the slide in Xylene_1 - 5 minutes
- 3 Immerse the slide in Xylene_2 - 6 quick immersions

HYDRATE

- 4 Immerse the slide in 100% alcohol_1 - 6 quick immersions
- 5 Immerse the slide in 100% alcohol_2 - 6 quick immersions
- 6 Immerse the slide in 96% alcohol - 6 quick immersions
- 7 Immerse the slide in 70% alcohol - 6 quick immersions
- 8 Immerse the slide in 50% alcohol - 6 quick immersions
- 9 Immerse the slide in Distilled water (H₂O) - 2 minutes

STAINING

- 10 Apply Harris Hematoxylin - according to preparation
- 11 Run the slide under tap water (H₂O) - 5 minutes



DIFFERENTIATE HEMATOXYLIN

- 12 Apply Acid alcohol - 2 minutes. Acid alcohol is made by adding 0.3 ml of HCl to 250 ml of 70° alcohol.
- 13 Run the slide under tap water (H₂O) - 5 minutes

BLUEING

- 14 Apply NaHCO₃ - 1 minute. Add one tablespoon of NaHCO₃ to 250 ml of distilled water (H₂O).
- 15 Run the slide under tap water (H₂O) - 5 minutes

COUNTERSTAINING

- 16 Immerse the slide in 96% alcohol - 3 minutes.
- 17 Apply Eosin - according to preparation

DEHYDRATE

- 18 Immerse the slide in 96% alcohol - 6 quick immersions
- 19 Immerse the slide in 96% alcohol - 6 quick immersions
- 20 Immerse the slide in 100% alcohol - 6 quick immersions
- 21 Immerse the slide in 100% alcohol - 6 quick immersions



CLEAR

- 22 Immerse the slide in Xylene - until the preparations are clear. Leave in xylene until mounting.

Acknowledgements

The Hematoxylin and Eosin (H&E) Staining for Formalin-Fixed Paraffin-Embedded Human Lymph Node Tissues Protocol was done by Yale Pathology Tissue Services (YPTS).