OmniStain H&E

*** Incubate at 20–25°C unless otherwise specified.*
*** Use fresh solution for each repeat. Keep the specimen fully submerged at all times and agitate on an orbital shaker at 100 rpm, and place the tube horizontally to maximize fluid flow unless otherwise specified.*

Compatible with formalin-fixed tissues. For FFPE tissues, retrieve antigens with **OmniStain REZCUE** *first.*

If you plan to do 3D immunostaining on the same specimen, refer to the **OmniStain H&E + IMMUNO** *protocol.*

Tissue pretreatment

1. Place the tissue in a 50 mL conical tube.

2. Dehydrate in 25 mL 50% v/v methanol in H_2O 2 x 30 min, then in 25 mL 100% methanol 3 x 30 min.

3. Delipidate in 25 mL 2:1 v/v dichloromethane:methanol (2 x 2 hours) in a fume hood.

4. Wash in 25 mL 100% methanol (3 x 15 min).

5. Wash in 5 mL **OmniStain H&E pre-wash solution** (2 x 30 min), agitate with the tube upright.

H&E-like Staining

6. Prepare 0.4% w/v eosin Y stock solution in water.

7. Prepare a 1 mM or 5 mM nucleic acid dye stock solution for hematoxylin-like staining in DMSO.
   - Compatible dyes include BOBO-1, BOBO-3, POPO-1, TOTO-3, and YOYO-3.

8. Prepare 5 mL of final **OmniStain H&E staining solution** per specimen by adding the following dyes:
   - eosin at 0.004% w/v final concentration (i.e. 1:100 dilution),
   - nucleic acid dye at 5 µM final concentration (i.e. 1:1000 dilution for a 5 mM stock or 1:200 for a 1 mM stock).

9. Stain in 5 mL **OmniStain H&E staining solution** for 6-18 hours with the tube upright.
   - If necessary, add more staining solution with the same dye concentrations to cover the tissue.
   - Protect the tissue from light from this step onwards.

Washing and Tissue clearing

10. Wash in 25 mL 50% v/v methanol in H_2O, 2 x 15 min.

11. Wash in 5 mL **OmniStain H&E post-wash solution** (2 x 30 min) and agitate with the tube upright.

12. Wash in 25 mL 100% methanol (3 x 30 min).

13. Incubate in 20 mL **Omni CLEAR** or BABB for 30 min. OmniStain H&E accelerates tissue clearing.

14. Replace with 20 mL fresh **Omni CLEAR** or BABB, incubate for 2 hours or until optically clear.

Have questions? Contact us at sales@illumos.co or visit www.illumos.co

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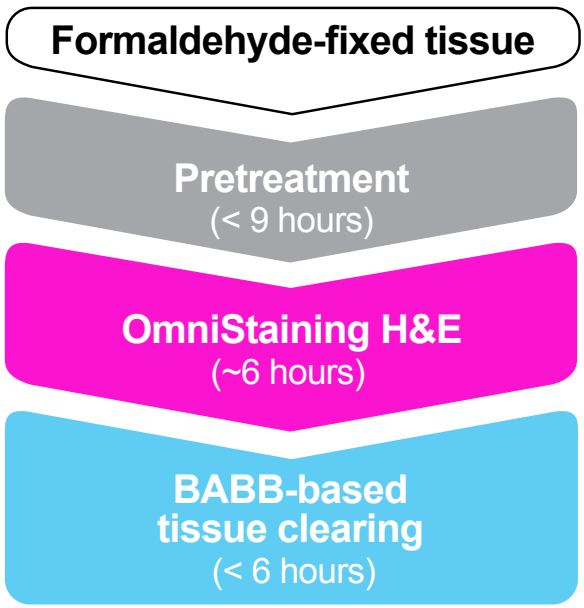
OmniStain H&E

Summary



Materials provided

- **1x OmniStain H&E pre-wash solution**
- **1x OmniStain H&E staining solution**
- **1x OmniStain H&E post-wash solution**

Materials not provided but required

- Eosin Y, disodium salt (CAS No. 17372-87-1)
- Nucleic acid dye. Compatible dyes are BOBO-1, BOBO-3, POPO-1, TOTO-3 and YOYO-3
- **Omni CLEAR** or 1:2 v/v Benzyl alcohol: benzyl benzoate (BABB)
- Dichloromethane
- Methanol
- DMSO
- Deionized water
- Shaker
- Fume hood
- 50 mL polypropylene conical tubes
- 2.5 µL to 1000 µL adjustable micropipettes with disposable tips

Introduction

- OmniStain H&E is a **rapid 3D H&E-like** fluorescence staining method, designed for human tissues.
- It supports deep and uniform penetration with excellent tissue clearing for light-sheet and confocal microscopy.
- The **protocol takes ~24 hours for 3 cm x 2 cm x 5 mm-thick tissues**, without specialized equipment.
- The workflow has a turnaround time comparable to routine clinical histopathology processing, making it suitable for clinical specimens and research applications.
- The specimen is compatible with downstream routine histology (H&E, special stains, IHC), whole-genome sequencing (WGS), RNA sequencing, spatial transcriptomics, and mass spectrometry-based proteomics, following validated procedures.
- If applied to transgenic animal tissues, OmniStain H&E quenches fluorescent proteins.

Notes and Tips

- To apply **OmniStain H&E** on **FFPE specimens**, perform **OmniStain REZCUE** before beginning this staining and clearing protocol.
- Use **Omni CLEAR** or BABB for the best clearing results for human tissues.
- If you want to add immunostaining, follow the **OmniStain H&E + IMMUNO protocol.**
- If you are working with difficult samples, contact us at sales@illumos.co; we typically reply within 24 hours.

Video and resources:
www.illumos.co/a-quick-guide-to-omnistain/