APR 03, 2024

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.kqdg3242qv25/v1

**Protocol Citation:** Kyu Sang Han, Pei-Hsun Wu, Joel Sunshine, Ashley Kiemen, Miklhail James, Sashank Reddy, Denis Wirtz 2024. Tissue H&E Staining | HuBMAP | JHU-TMC. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg3242qv25/v1



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

# Tissue H&E Staining | HuBMAP | JHU-TMC

Kyu Sang Han[1], Pei-Hsun Wu[1], Joel Sunshine[2], Ashley Kiemen[2], Miklhail James[2], Sashank Reddy[2], Denis Wirtz[1,2]

[1]Johns Hopkins University; [2]Johns Hopkins Medicine

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

TMC - Johns Hopkins University

Kyu Sang Han
Johns Hopkins University

ABSTRACT

The following are guidelines that will have an effective staining window of 2 to 5 minutes. Developed as progressive stains, the intensity of nuclear staining will increase as the time increases. The hematoxylin and eosin stains will have an expected throughput of 2,000-2,500 slides per 500mL bottle. Actual results may vary from lab to lab depending on the staining equipment used, control of carry over into each solution, and length of time stains are left exposed. As a general rule, we recommend changing the hematoxylin and eosin stains once per week if throughput has not been reached. Clarifying and Bluing solutions should be changed more often.

The Leica autostainer is a single fully automated integrated stainer used for standard H&E staining and operates by progressing each slide through a series of chemical changes that first deparaffinize and then stains the tissue slides for histologic review. To use, the machine is powered on and the covers are removed from the staining vessels every morning and the hematoxylin is filtered daily. The daily number of slides stained is added to the log sheet beside the stainer, and all the solutions are changed when the slide tally reaches 250. At the end of the day, the staining vessels are then covered and the machine is powered off

PROTOCOL REFERENCES

Avantik, April 3 2024, Optik Type 1 | Avantik (avantik-us.com)

**Created:** Mar 29, 2024

**Last Modified:** Apr 03, 2024

**PROTOCOL integer ID:** 97520

**Keywords:** H&E, histology

**Funders Acknowledgement:**
Institute of Arthritis and Musculoskeletal and Skin Diseases
Grant ID: U54AR081774
National Cancer Institute
Grant ID: U54CA143868

MATERIALS

The Leica autostainer
Xylene
Ethanol
Hematoxylin
Clarifier
Bluring reagent
Eosin

## Before staining

1 Bake unstained slides for 30-60 minutes at 60 degrees Celsius.

## Dewaxing

2 The following steps require setting up multiple stations with different solutions.

3 Set 2 stations with Xylene. Submerge the unstained slide for 3 minutes in each station.

4 Set 2 stations with 100% ethanol. Submerge the slide for a minute in each station.

5 Set a station with 95% ethanol. Submerge the slide for a minute in each station.

## H&E staining

6 Wash the slide in running warm water for a minute

7 Submerge the slide in Optik Hematoxylin for 3 minutes

8 Wash the slide in running warm water for a minute

9 Submerge the slide in Optik Aqueous Clarifier Type 1 for a minute

10 Wash the slide in running warm water for a minute

11 Submerge the slide in Optik Bluing Solution Type 1 for a minute

12 Wash the slide in running warm water for a minute

13 Submerge the slide in Optik Eosin Y Type 1 for a minute

14 Set a station with 95% ethanol. Submerge the slide for a minute in each station.

15 Set 2 stations with 100% ethanol. Submerge the slide for a minute in each station.

16 Set 3 stations with Xylene. Submerge the dewaxed unstained slide for a minute in each station.

17 **Final Step:** Mount and Coverslip with Covermount