# Hematoxylin & Eosin Staining of Tissue Section

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| Category: | **Materials Handling and Documentation** | | |
| SOP number: | 04.010 | Version | 1.0 |
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| Approved by: | Dr. Alaa A. AlMasud | Approved On: | |

## 1.0 PURPOSE

The purpose of this SOP is to establish standardized procedures for the Hematoxylin and Eosin (H&E) staining of tissue sections at Nourah's Tissue Biobank. H&E staining is a critical technique used to visualize the morphological details of tissue samples, aiding in histological analysis.

## 2.0 SCOPE

This SOP applies to all personnel involved in the staining of tissue sections using Hematoxylin and Eosin within Nourah's Tissue Biobank. It covers the steps required to prepare, stain, and document tissue sections using the LabVantage LIMS system.

## 3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of Nourah's Tissue Biobank members

| Biobank Personnel | Responsibility |
|---|---|
| Pathologist/Pathologist Assistant | Responsible for reviewing stained slides for quality assurance and diagnostic purposes. |
| Laboratory Technicians | Responsible for preparing tissue sections, performing H&E staining according to this SOP, and ensuring the proper handling, labeling, and documentation of stained slides. |
| Biobank Manager | Responsible for overseeing the H&E staining process, ensuring compliance with this SOP, and addressing any issues that arise. |
| Quality Assurance (QA) Officer | Responsible for auditing the H&E staining process, ensuring adherence to protocols and regulatory requirements. |

## 4.0 MATERIALS, EQUIPMENT, AND FORMS

Listing of the materials, equipment, and forms being used to achieve the goals of the SOP, this list will mainly contain necessary materials and, or recommendations that may be substituted by alternative or equivalent materials more suitable at the time of testing.

| Material to be used | Site |
|---|---|
| Tissue sections mounted on glass slides | |
| Hematoxylin solution | |
| Eosin solution | |
| Deionized water | |
| Alcohol solutions (graded ethanol series) | |
| Xylene or xylene substitute | |
| Coverslips and mounting medium | |
| Staining racks | |
| Coplin jars or staining dishes | |
| Automated or manual staining setup | |
| Forceps | |
| Labels and markers | |
| PPE (gloves, lab coat, face mask) | |
| Biohazard waste containers | |

## 5.0 POTENTIAL HAZARDS

In this part of the SOP, we explain the potential hazards from chemicals and methodologies used in this procedure. It will also contain information on how to handle these chemicals and the level of biosafety

| Material | Safety and handling |
|---|---|
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## 6.0 PROCEDURES

This section outlines the steps involved in preparing tissue sections, performing H&E staining, and documenting the process in Nourah's Tissue Biobank. These procedures ensure consistent and high-quality staining for histological analysis.

### 6.1 PREPARATION OF SLIDES

1. Ensure that tissue sections are properly fixed, processed, and embedded in paraffin prior to sectioning.
2. Use a microtome to cut thin sections (typically 4-5 micrometers thick) from paraffin blocks and mount the sections on glass slides.
3. Place the section in the oven to bake at 60°C for 20-30 minutes
4. Place the slides in xylene for 2-3 minutes to remove the paraffin from the tissue sections.
5. Transfer the slides through two additional changes of xylene to ensure complete deparaffinization.
6. Rehydrate the tissue sections by passing the slides through a series of graded ethanol solutions: 100% ethanol for 2 minutes, 95% ethanol for 2 minutes, and 70% ethanol for 2 minutes.

7. Rinse the slides in deionized water for 2 minutes to prepare them for staining.
8. Immerse the slides in Harris Hematoxylin solution for 2 minutes to achieve nuclear staining.
9. Rinse the slides in running tap water for 2 minutes or 10 dips in two different changes of tap water to remove excess hematoxylin.
10. Dip the slides in acid alcohol (1% hydrochloric acid in 70% ethanol) for 5-10 seconds to differentiate and remove excess hematoxylin, ensuring clear nuclear detail.
11. Immediately rinse the slides in running tap water for 1-2 minutes to stop the differentiation process.
12. Place the slides in ammonia water for 1-2 minutes to enhance the blue color of the nuclei (bluing).
13. Rinse the slides again in running tap water for 2 minutes to prepare them for eosin staining.
14. Immerse the slides in Eosin solution for 1-2 minutes to stain the cytoplasm and extracellular matrix pink.
15. Rinse the slides briefly in deionized water to remove excess eosin and achieve the desired intensity.
16. Dehydrate the slides by passing them through graded ethanol solutions: 70% ethanol for 1 minute, 95% ethanol for 1 minute, and 100% ethanol for 2 minutes.
17. Ensure that the slides are fully dehydrated to prevent water from interfering with the mounting medium.
18. Clear the slides by immersing them in xylene or a xylene substitute for 2-3 minutes.
19. Repeat the clearing step with a fresh xylene solution to ensure complete removal of ethanol.
20. Apply a coverslip to each slide using a mounting medium suitable for xylene-cleared slides.
21. Ensure that the coverslip is free of air bubbles and is securely attached to the slide.
22. Label each slide with the necessary information, including patient identifier, tissue type, date, and any specific staining details.
23. Verify that the labeling is legible and securely attached to the slides.
24. Enter the details of the H&E-stained slides into the LabVantage LIMS system immediately after staining.
25. Document the staining protocol, any observations regarding the quality of the staining, and the storage location of the slides.

## 6.2 QUALITY CONTROL AND SLIDE EVALUATION

1. The pathologist or pathologist assistant reviews the stained slides under a microscope to assess the quality of the staining and tissue morphology.
2. Record any issues or observations that may affect the utility of the slides.
3. Document the results of the slide evaluation in the LIMS, noting any corrective actions taken if the staining did not meet the required standards.

## 6.3 STORAGE AND TRANSPORT

1. Store the stained slides in a designated slide archive at room temperature, ensuring they are properly labeled and recorded in the LIMS.
2. Update the storage location and conditions in the LabVantage LIMS system for accurate tracking.
3. Archive the stained slides in slide storage boxes or cabinets, organized by patient identifier or case number for easy retrieval.

## 6.4 INCIDENT MANAGEMENT

1. In case of any deviations from the SOP (e.g., temperature excursions, delays), document the incident and notify the Biobank Manager immediately.
2. Implement corrective actions as necessary to mitigate any impact on sample integrity.

3. Complete an incident report detailing the deviation, corrective actions taken, and any follow-up measures required.
4. Submit the report to the QA Officer for review and documentation.

## 7.0 REFERENCES

1. CTRnet SOPs "08.03.007 H & E Staining of Tissue Sections"
2. King Abdullah bin Abdulazizi University Hospital SOP "H&E Staining"
3. Declaration of Helsinki.
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4. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
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5. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
   http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories
6. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
   http://bioethics.georgetown.edu/nbac/hbm.pdf
7. US National Biospecimen Network Blueprint
   http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

## 8.0 REVISION HISTORY

| SOP No. | Date Revised | Author | Summary |
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## 9.0 APPENDICES