🔬 H&E Staining Protocol

Hematoxylin and Eosin Staining for Tissue Sections

Protocol Overview

Hematoxylin and Eosin (H&E) staining is the most widely used staining method in histology. Hematoxylin stains nuclei blue-purple, while eosin stains cytoplasm and extracellular matrix pink.

⏱️ Duration

Paraffin sections: ~1-1.5 hours

Cryo sections: ~1-1.5 hours

🎯 Key Steps

1. Deparaffinization (paraffin) or Rehydration (cryo)
2. Hematoxylin staining
3. Eosin staining
4. Dehydration and mounting

> 💡 **What it stains:**
>
> - **Hematoxylin:** Nuclei (blue-purple)
> - **Eosin:** Cytoplasm, collagen, muscle fibers (pink-red)

Required Materials

Reagents

- Xylene (3 coplin jars)
- 100% Ethanol (3 coplin jars)
- 95% Ethanol
- 70% Ethanol
- 50% Ethanol
- 20% Ethanol
- Distilled water (ddH_2O)
- Hematoxylin solution
- Eosin solution
- 2% Acetic acid
- Bluing solution
- Histomount mounting medium

Equipment

- Coplin jars (for staining solutions)
- Slide rack
- Timer
- Running water source (warm recommended)
- Coverslips
- Slide storage box
- Fume hood (for xylene steps)

Select Sample Type:

Paraffin Sections | Cryo Sections

Paraffin Section Protocol

1 Deparaffinization

- [ ] Xylene - 5 minutes
- [ ] Xylene - 5 minutes
- [ ] Xylene - 5 minutes

2 Hydration

- [ ] 100% EtOH - 2 minutes
- [ ] 100% EtOH - 2 minutes
- [ ] 95% EtOH - 2 minutes
- [ ] 70% EtOH - 1 minute
- [ ] 50% EtOH - 1 minute
- [ ] 20% EtOH - 1 minute
- [ ] Running H_2O - 4 minutes

3 Hematoxylin Staining

- [ ] Hematoxylin - few seconds to 1 minute

  ⚠️ Time depends on solution age. Typically 20-45 seconds for fresh, up to 1 min for older solutions

- [ ] Running H_2O - 1.5 minutes (dip slides)
- [ ] 2% Acetic acid - 1.5 minutes (dip slides)

- [ ] Running H_2O - 1 minute (dip slides)
- [ ] Bluing solution - 1.5 minutes (dip slides)
- [ ] Running H_2O - 1 minute (dip slides)

4 Eosin Staining

- [ ] Eosin - 1 minute (dip slides)
- [ ] Running H_2O - 1 minute (dip slides)

5 Dehydration

- [ ] 70% EtOH - 1 minute
- [ ] 95% EtOH - 1 minute
- [ ] 100% EtOH - 1 minute
- [ ] 100% EtOH - 2 minutes
- [ ] 100% EtOH - 2 minutes

6 Clearing

- [ ] Xylene - 5 minutes
- [ ] Xylene - 5 minutes

- [ ] Xylene - 5 minutes

7 Mounting and Storage

- [ ] Add Histomount mounting medium and place coverslip
- [ ] Store sections at room temperature

Cryo Section Protocol

1 Rehydration

- [ ] Running H_2O - 4-5 minutes
- [ ] 20% EtOH - 1 minute
- [ ] 50% EtOH - 1 minute
- [ ] 70% EtOH - 1 minute
- [ ] 95% EtOH - 2 minutes
- [ ] 100% EtOH - 2 minutes
- [ ] 100% EtOH - 2 minutes
- [ ] 95% EtOH - 2 minutes

- [ ] 70% EtOH - 1 minute
- [ ] 50% EtOH - 1 minute
- [ ] 20% EtOH - 1 minute
- [ ] Running H_2O - 4-5 minutes

2 Hematoxylin Staining

- [ ] Hematoxylin - 5 minutes
- [ ] Running H_2O - 1.5 minutes (dip slides)
- [ ] 2% Acetic acid - 1.5 minutes (dip slides)
- [ ] Running H_2O - 1 minute (dip slides)
- [ ] Bluing solution - 1.5 minutes (dip slides)
- [ ] Running H_2O - 1 minute (dip slides)

3 Eosin Staining

- [ ] Eosin - 2 minutes (dip slides)
- [ ] Running H_2O - 1 minute (dip slides)

4 Dehydration

- [ ] 70% EtOH - 1 minute
- [ ] 95% EtOH - 1 minute
- [ ] 100% EtOH - 1 minute
- [ ] 100% EtOH - 2 minutes
- [ ] 100% EtOH - 2 minutes

5 Clearing

- [ ] Xylene - 5 minutes
- [ ] Xylene - 5 minutes
- [ ] Xylene - 5 minutes

6 Mounting and Storage

- [ ] Add Histomount mounting medium and place coverslip
- [ ] Store sections at room temperature

Tips & Notes

Hematoxylin Timing

The hematoxylin staining time depends on how many times the solution has been used. Fresh hematoxylin may require only 20-45 seconds, while older solutions may

need up to 1 minute. Always monitor staining intensity under a microscope if possible.

Use Warm Water

Using warm running water for rinses helps improve staining quality and reduces background.

Dipping vs. Immersion

When the protocol says "dip slides," gently move slides up and down in the solution. This agitation helps ensure even staining and thorough washing.

Work in Fume Hood

Always work with xylene in a fume hood. Xylene fumes are toxic and should not be inhaled.

Bluing Solution Purpose

The bluing solution helps develop the blue-purple color of hematoxylin staining. This step is crucial for optimal nuclear contrast.

Troubleshooting Guide

Weak or No Nuclear Staining

Possible Causes:

- Hematoxylin solution is old or exhausted
- Insufficient staining time
- Inadequate bluing

Solutions:

- Use fresh hematoxylin or increase staining time
- Ensure proper bluing (pH adjustment)
- Check that fixation was adequate

Overstained (Too Dark)

Possible Causes:

- Hematoxylin staining time too long
- Eosin staining time too long

Solutions:

- Reduce staining time in future runs
- Can differentiate in acid alcohol if caught early

Weak Eosin Staining

Possible Causes:

- Eosin solution is old
- Insufficient staining time
- Too much water carryover from previous step

Solutions:

- Use fresh eosin
- Increase staining time
- Ensure proper draining between steps

Precipitate on Slides

Possible Causes:

- Staining solutions are dirty or contaminated
- Insufficient washing
- Slides not completely deparaffinized

Solutions:

- Filter staining solutions
- Change xylene and ethanol solutions regularly
- Ensure thorough washing between steps

Tissue Falls Off Slides

Possible Causes:

- Slides not properly coated or charged
- Insufficient drying after sectioning
- Poor tissue adhesion

Solutions:

- Use positively charged or coated slides
- Bake slides at 60°C for 30-60 minutes before staining
- Ensure gentle agitation during washes

⚠️

Safety Information

- Always work with xylene in a fume hood - fumes are toxic
- Wear appropriate PPE (lab coat, gloves, safety glasses)
- Hematoxylin can stain skin - wear gloves at all times
- Dispose of xylene and ethanol as hazardous waste
- Keep all staining solutions away from open flames
- Ensure adequate ventilation in the staining area

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About This Protocol

Standard Hematoxylin and Eosin (H&E) staining protocol for both paraffin-embedded and cryosectioned tissue samples.

Quick Links

Protocol Overview

Troubleshooting

Contact

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