

Protocol

Hematoxylin and Eosin Staining of Tissue and Cell Sections

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This protocol was adapted from "Preparation of Cells and Tissues for Fluorescence Microscopy," Chapter 4, in *Basic Methods in Microscopy* (eds. Spector and Goldman). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2006.

INTRODUCTION

Hematoxylin and eosin (H&E) stains have been used for at least a century and are still essential for recognizing various tissue types and the morphologic changes that form the basis of contemporary cancer diagnosis. The stain has been unchanged for many years because it works well with a variety of fixatives and displays a broad range of cytoplasmic, nuclear, and extracellular matrix features. Hematoxylin has a deep blue-purple color and stains nucleic acids by a complex, incompletely understood reaction. Eosin is pink and stains proteins nonspecifically. In a typical tissue, nuclei are stained blue, whereas the cytoplasm and extracellular matrix have varying degrees of pink staining. Well-fixed cells show considerable intranuclear detail. Nuclei show varying cell-type- and cancer-type-specific patterns of condensation of heterochromatin (hematoxylin staining) that are diagnostically very important. Nucleoli stain with eosin. If abundant polyribosomes are present, the cytoplasm will have a distinct blue cast. The Golgi zone can be tentatively identified by the absence of staining in a region next to the nucleus. Thus, the stain discloses abundant structural information, with specific functional implications. A limitation of hematoxylin staining is that it is incompatible with immunofluorescence. It is useful, however, to stain one serial paraffin section from a tissue in which immunofluorescence will be performed. Hematoxylin, generally without eosin, is useful as a counterstain for many immunohistochemical or hybridization procedures that use colorimetric substrates (such as alkaline phosphatase or peroxidase). This protocol describes H&E staining of tissue and cell sections.

RELATED INFORMATION

Preparation of tissue sections is described in the **Cutting Sections of Paraffin Embedded Tissues** (Fischer et al. 2008). Two other stains are also versatile: Giemsa staining, particularly for cells fixed by air-drying, and the Papanicolaou stain, which provides a transparent, highly detailed stain useful for thick cellular samples. For details regarding Giemsa and Papanicolaou stains, refer to Sheehan and Hrapchak (1980) and Carson (1997).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

Cells or tissue of interest on microscope slide (see Step 1.i)
Eosin Y (1% aqueous solution; EM Diagnostic Systems)

Ethanol (95%, 100%)

<!--Methanol or Flex alcohols (Richard-Allan Scientific) can be used instead of ethanol (see Step 5).-->

<!--Hematoxylin, Mayer's (Sigma)

Mayer's hematoxylin is the easiest to use and is compatible with most colorimetric substrates.

Mounting medium (Canada Balsam, Sigma C1795)

Use glycerol or other aqueous mounting media if alcohols cannot be used (see Step 7).

<!--Xylene

Equipment

Coplin jar (Fisher Scientific)

Coverslips

METHOD

1. Hydrate the cells or tissue:
 - i. Use a microscope slide bearing cryosections or rehydrated tissue sections (see Step 12 in **Cutting Sections of Paraffin Embedded Tissues**) (Fischer et al. 2008) fixed in either alcohol or an aldehyde-based fixative.
 - ii. Immerse the slide for 30 sec with agitation by hand in H₂O.
A rinse in H₂O is important; hematoxylin precipitates with salts and buffers. The staining can be performed after immunohistochemical or hybridization reactions with nonfluorescent detection systems.
2. Dip the slide into a Coplin jar containing Mayer's hematoxylin and agitate for 30 sec.
3. Rinse the slide in H₂O for 1 min.
Estimate the staining intensity at this point, and repeat Steps 2 and 3 if necessary.
4. Stain the slide with 1% eosin Y solution for 10-30 sec with agitation.
5. Dehydrate the sections with two changes of 95% alcohol and two changes of 100% alcohol for 30 sec each.
Some colorimetric substrates dissolve in alcohol.
6. Extract the alcohol with two changes of xylene.
If using plastic slides or staining in plastic culture dishes, do not use xylene or xylene-based mounting media, because they dissolve plastics.
7. Add one or two drops of mounting medium and cover with a coverslip.
If alcohols cannot be used, mount the coverslip with glycerol or other aqueous mounting media.

REFERENCES

- Carson, F.L. 1997. *Histotechnology: A self-instructional text*, 2nd ed. ASCP Press, Chicago. doi: 10.1101/pdb.prot4987.
- Fischer, A.H., Jacobson, K.A., Rose, J., and Zeller, R. 2008. Cutting sections of paraffin embedded tissues. *CSH Protocols* (this issue)
- Sheehan, D.C. and Hrapchak, B.B. 1980. *Theory and practice of histotechnology*, 2nd ed. Battelle Press, Columbus, OH.



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