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# Hematoxylin and Eosin Staining (H&E) - Pregnant Female Reproductive TMC

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**We use this protocol and it's working**

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## Abstract

Here we describe the protocol for hematoxylin and eosin (H&E) histological staining employed by the UC San Diego Human & Animal Tissue Technology Center. H&E is used to distinguish nuclear and cytoplasmic cellular compartments and other cellular structures, as well as provide insights into overall patterns present within a given tissue.

## Guidelines

Steps involving xylene or alcohol should be conducted in a fume hood; for the sake of simplicity, the author conducts all steps except water rinses in a fume hood.

## Materials

A	B
100% reagent alcohol	Fisher Chemical A962P-4
hematoxylin	Epredia 7212
eosin Y with phloxine	Epredia 71304
xylene	Fisher Chemical X5-4
define solution (Clarifier 2)	Epredia 7402
bluing solution	Epredia 7301
tap water	
coverslip, 24 × 30mm #1.5	VWR 48404-467
slides	Fisher Scientific 12-550-15
coverslip mounting solution	Cytoseal XYL 8312-4
Sakura 24 slide holder	Tissue-Tek 4465
slide staining dishes	Tissue-Tek 4456 or 4457

## Troubleshooting

## Safety warnings

- !
  - **XYLENE:** Flammable, toxic, avoid contact with eyes and skin
  - **100% REAGENT ALCOHOL:** Flammable, poisonous, avoid contact with eyes and skin
  - **HEMATOXYLIN:** Flammable, harmful vapors, poisonous, avoid contact with eyes, skin and clothing
  - **EOSIN:** Harmful vapor, avoid contact with eyes, skin, and clothing
  - **BLUING REAGENT:** Avoid contact with eyes and skin
  - **DEFINE REAGENT:** Avoid contact with eyes



## Dry slides

8h

- 1 Place slides into a slide holder and air dry slides overnight prior to staining.

8h

## Deparaffinization

31m

- 2 Prepare six slide staining dishes
  - Xylene (2)
  - 100% reagent alcohol (3)
  - Tap water (1)
- 3 Submerge slides in the first xylene dish for 10 minutes. Gently mix up and down 3-5 times during incubation to facilitate wax removal.
- 4 Remove slides and submerge in the second xylene dish for 5 minutes. Gently mix up and down 3-5 times during incubation to facilitate wax removal.
- 5 Remove slides, draining off as much xylene as possible, and submerge in the first reagent alcohol dish for 2 minutes. Repeat twice more, removing and submerging into the next reagent alcohol dish, for a total of three submersions in reagent alcohol.
- 6 Remove slides, submerge slides in water dish, remove slides, discard water, and refill dish with fresh tap water. Repeat 2 or 3 more times.

5m

10m

5m

6m

5m

## Staining

1d 0h 59m

- 7 Prepare eleven staining dishes:
  - Hematoxylin (1)
  - Define solution (1)
  - Bluing solution (1)
  - Eosin-Y with phloxine (1)
  - 100% reagent alcohol (3)
  - Xylene (3)
  - Tap water (1)
- 8 Begin staining by submerging slides in hematoxylin dish for 1 minute.
- 9 Remove slides from stain and rinse with running tap water until water runs clear, approximately 1 minute.

1m

1m



- 10 Submerge slides in define solution for 1 minute. 1m
- 11 Remove slides, submerge slides in water dish, remove slides, discard water, and refill dish with fresh tap water. Repeat 2 or 3 more times. 5m
- 12 Submerge slides in bluing solution for 1 minute. 1m
- 13 Remove slides, submerge slides in water dish, remove slides, discard water, and refill dish with fresh tap water. Repeat 2 or 3 more times. 5m
- 14 Submerge slides in eosin for 1 minute. 1m
- 15 Remove slides, submerge slides in water dish, remove slides, discard water, and refill dish with fresh tap water. Repeat 2 or 3 more times. 5m
- 16 Air dry slides in slide holder for 15 minutes. 15m
- 17 Submerge slides in the first **reagent alcohol** dish for 1 minute. Repeat this step twice more, removing and submerging into the next reagent alcohol dish, for a total of three submersions in reagent alcohol. 3m
- 18 Air dry slides in slide holder for 15 minutes. 15m
- 19 Remove slides and submerge in the first **xylene** dish for 1 minute. Repeat this step twice more, removing and submerging into the next xylene dish, for a total of three submersions in xylene. 3m
- 20 Drain excess xylene and apply coverslip mounting medium. 3m
- 21 Apply coverslip to the slides and let air dry for 1 or 2 days. 1d