

Hematoxylin & Eosin Staining of Brain Sections

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MWS

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These staining solutions can be purchased from Fisher—

Harris Modified Hematoxylin (0.5%), Cat no. SH26-500D, \$41.63 per 500 mL

Eosin Yellowish Solution (1% w/v), Cat no. SE23-500D, \$38.80 per 500 mL

Note: Be VERY careful with hematoxylin—it's a nasty chemical. Always collect it as hazardous waste, and always wear gloves!

For paraffin sections:

1) Deparaffinize in a 3-minute series (3 min per solution) through the following:

Xylenes or HistoClear (HC)

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50% Xylenes or HC/ 50% ethanol (EtOH)

100% EtOH

100% EtOH

95% EtOH

70% EtOH

50% EtOH

dH₂O

For frozen sections:

1) Bring slides to room temp on bench 5-10 min

2) Rinse out OCT 5 min in PBS

Staining protocol:

1) Dip rehydrated slides 30 sec (for lighter stain) or 60 sec (for darker staining) in hematoxylin

2) Rinse with tap water until water runs clear

3) Dip slides 2 min in eosin Y

4) Rinse with tap water until water runs clear

5) Run slides through a quick dehydration series: 3 quick dips in each of the following solutions—50% EtOH

70% EtOH

95% EtOH

100% EtOH

100% EtOH

6) Incubate slides 1 min in 50% EtOH/ 50% HC (or xylenes)

7) Incubate slides 3 min in HC or xylenes

8) Repeat step 7

9) Mount with Permount

Results:

Nuclei and basic structures are blue; cytoplasm and acidic structures are pink or red.