

Immunocytochemistry Protocols *Hematoxylin and Eosin (H & E) Staining*

Hematoxylin stains negatively charged nucleic acids (nuclei & ribosomes) blue. Eosin stains proteins pink. Optimization may be necessary to achieve staining (hematoxylin may be diluted in H₂O and eosin may be diluted in ethanol as needed).

Reagents:

Harris Hematoxylin (EM Science)
Eosin Y (Sigma)
Ammonium Hydroxide, 0.3%
Ethanol
HistoClear or Xylene

Procedure:

- For paraffin-embedded sections:
Dewax either: 1) by baking at ~55-65°C for 45-60 min. Clean with either xylene (3x 5 min each) or HistoClear. Alternatively, you can dewax by 2) immersing in HistoClear (2x 5 min each).
- For frozen sections:
Allow sections to thaw briefly (just a few minutes). **Never let sections dry out.**

Following paraffin sections, rehydrate sections:

100% ethanol, two changes for 5 min each

70% ethanol, 1x for 5 min.

Water (running water for 5 min)

Stain as follows:

Hematoxylin (10 dips to 2 mins: optimize for your sections)

Water, 2x 1 min each (or until water looks clean)

Ammonium Hydroxide, 0.3%, 10-20 dips

Water, 2x 1 min each

(At this point, you can check the blue intensity under microscope; make sure you don't let sections dry out, repeat stain if necessary)

50% or 70% EtOH, 1 min.

80% EtOH, 1 min.

95% EtOH, 1 min.

100% EtOH, 2x 1 min each

Eosin-Y, (10 dips to 45 sec, optimize for your sections)

100% EtOH, 2x 1 min each (or until EtOH doesn't look pink)

HistoClear or Xylene, 2x 1 min

Permount

Cover slip