

HEMATOXYLIN AND EOSIN (H & E) STAINING PROTOCOL

TCH 11-25-03

Hematoxylin stains negatively charged nucleic acids (nuclei & ribosomes) blue. Eosin stains protein pink. Optimization may be necessary to achieve the best staining.

REAGENTS:

Harris Hematoxylin Hg⁺⁺-free, acidified, (Fisher Scientific)

Eosin-Y in 1% Alcohol (Fisher Scientific)

Ammonium Hydroxide, 0.3% (or 1% if the original stock is counted as 100%).

Ethanol

Xylene

10% Formalin (or 4% Formaldehyde, or 4% Paraformaldehyde[stable at 4C for one month]).

PRE-TREATMENT OF SAMPLES (SECTIONS):

For paraffin-embedded sections:

- 1) Bake the slides at 55-60C (in the oven or on a heating block) for 30-60min.
- 2) Deparaffinize (or dewax) the slides with xylene 3 times, 5 min each.
- 3) Rehydrate sections by immersing the slides into 100% ethanol (5min x2) >>> 70% ethanol (5min x1-2) >>> tap water for 5min.
- 4) Never let slides completely dry from this point.
- 5) Move to H & E staining steps.

For frozen sections:

- 1) Allow frozen sections to thaw briefly at room temperature.
- 2) Fix the sections with 10% Formalin (or 4% Formaldehyde, or 4% Paraformaldehyde).
- 3) Wash the fixed sections with PBS x2.
- 4) Never let slides completely dry from this point.
- 5) Move to H & E staining steps.

H & E STAINING PROCEDURE:

- 1) Immerse slides in filtered Harris hematoxylin for up to 2 min (optimization may be needed).
- 2) Rinse with water for 1 min x2 (or until water looks clean).
- 3) Immerse slides in 0.3% ammonium hydroxide (10-20 dips).
- 4) Rinse with water for 1 min x2.
(At this point, you can check the blue intensity under microscope; if too light, repeat hematoxylin stain; if too blue/dark, use 1% HCl to destain).
- 6) immerse slides in Eosin-Y (10 dips or <45 sec; optimize for your slides).
- 7) Wash with water, 3x 1min each
- 8) Dehydrate slides in the following order: 70% ethanol for 1min >>> 80% ethanol for 1min >>> 95% ethanol for 1 min x3 >>> 100% ethanol for 1 min x3 (or until ethanol doesn't look pink).
- 9) Immerse slides in xylene for 1 min, x2.
- 10) Add Permount and cover slips.

NOTE:

- 1) Meyer's Hematoxylin can also be used and does not need to be acidified. However, it stains less intensive. Good for counterstaining.
- 2) If hematoxylin stains too dark, 1% HCl can be used to destain.