

HEMATOXYLIN & EOSIN STAINING PROTOCOL FWRI HISTOLOGY

Note: Prior to staining, slides must be baked for at least two at a minimum temperature of 70° C to inhibit detachment of sections during the staining procedure.

STAINING PROCEDURE

1. Stain in Weigert's Hematoxylin, working solution, for 6 minutes.
2. Drain slides.
3. Differentiate in 70% Acid-Ethanol, pH 2.5, two changes, three quick dips each.
4. Differentiate in 70% Acid-DI, pH 2.5, two changes, three quick dips each.
5. Wash in running tap water for 15 minutes. Drain excess water from slides.
6. Stain in working Eosin-Phloxine solution for 2 minutes.
7. Drain slides.
8. Differentiate and dehydrate in 95% ethanol, two changes, 30 seconds each.
9. Completely dehydrate in 100% ethanol, three changes, three quick dips each.
10. Clear in HistoClear, two changes, one minute each.
11. Fresh HistoClear and coverslip with mounting medium.

RESULTS

Nuclei – deep blue-violet

Chromatin – blue-black

Nucleoli – varying shades of orange-pink to red-violet, depending on cell type.

Cytoplasm – varying shades of pink and orange

Collagen – pale pink

Muscle – deep pink