RICCA CHEMICAL COMPANY®
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Hematoxylin and Eosin (H&E) Staining (Regressive)

Reagents Needed:

Hematoxylin Stain. Use one of the following:	
Hematoxylin Stain, Modified Harris	RICCA CHEMICAL COMPANY Cat. No. 3530
Hematoxylin Stain, Modified Harris	RICCA CHEMICAL COMPANY Cat. No. 3536
Hematoxylin Stain, Modified Harris	RICCA CHEMICAL COMPANY Cat. No. 3537
Eosin Y Counterstain. Use one of the following:	
Eosin Y Stain, 0.25% (w/v) in 57% (v/v) Alcohol, Acidified Working Solution	RICCA CHEMICAL COMPANY Cat. No. 2845
Eosin Y Stain, 1% (w/v) Alcoholic Stock Solution *If this Stock Solution is used, prepare the Working Solution by mixing 25 mL of Eosin Y Stock Solution, 75 mL of purified Water, and 0.5 mL of Glacial Acetic Acid immediately before use.*	RICCA CHEMICAL COMPANY Cat. No. 2850
Acid Alcohol, 1% HCl in 70% Alcohol	RICCA CHEMICAL COMPANY Cat. No. 245
Scott's Bluing Reagent	RICCA CHEMICAL COMPANY Cat. No. 6697

Recommended Method:

1. Fix tissue samples in the usual manner. All histological fixatives are suitable.
2. Prepare approximately 6 µm thick Paraffin Wax embedded tissue sections in the usual manner.
3. Deparaffinize and hydrate to water:
   a. Xylene – 2 x 2 minutes.
   b. Absolute (100%) Alcohol – 2 x 1 minute.
   c. Alcohol, 95% - 2 x 1 minute.
   d. Alcohol, 70% - 1 x 1 minute.
4. If a fixative containing Mercuric Chloride (such as Zenker's Fixative Solution) was employed, remove any Mercuric Chloride Crystals as follows:
   a. Gram's Iodine or Lugol's Iodine - 15 minutes
   b. Tap Water - 4 dips
   c. 5% Aqueous Sodium Thiosulfate (Hypo) - 3 minutes
5. Tap water - 10 minutes.
6. Hematoxylin - 15 minutes.
7. Tap water - 4 dips.
8. Acid Alcohol - 3 to 10 quick dips.
9. Check the differentiation with a microscope. Nuclei should be distinct and nuclear substances should be clearly visible, showing some metachromatic properties. The background Cytoplasm should be very light or preferably colorless. Continue differentiating in Acid Alcohol if necessary.
10. Tap water - 4 dips (very brief wash).
11. Scott's Bluing Reagent - 3 to 5 dips (until bright blue).
12. Wash in running, lukewarm, tap water - 15 to 20 minutes.
13. Eosin Y Working Solution - 15 seconds to 2 minutes. For even staining results, dip slides several times before allowing them to sit in the Eosin Y.
14. Dehydrate, clear, and mount:
    a. Alcohol, 95% - 2 x 1 minute.
    b. Absolute (100%) Alcohol – 2 x 1 minute.
    c. Check for proper removal of excess Eosin Y with a microscope. Wash again in Absolute Alcohol if necessary.
    d. Xylene – 3 x 2 minutes.
    e. Drain on filter paper and mount in Permount® (Fisher Scientific Company), or equivalent, before drying.

Satisfactory Staining Results:

A well-stained tissue sample will have blue nuclei with some metachromasia (from the Hematoxylin) and will have various shades of pink cytoplasm (from the Eosin) identifying different tissue components. Erythrocytes, if present, will usually stain a light orange color, and collagen, if present, will usually stain a salmon pink color.

This is a typical staining procedure. These reagents may be suitable for other staining procedures. Consult staining reference books or standard operating procedures for other suitable uses of these products.