



# KIDDRC Histology Services Protocols

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## Eriochrome(Solochrome) Cyanine(EC)

Stain for myelin safety

### **Safety recommendations:**

**The chemicals identified with \*are toxic.**

**Use gloves, lab coat, and safety goggles and perform staining in a fume hood.**

### **Solutions:**

#### **10% Iron Alum(Ferric Ammonium Sulfate)**

Ferric Ammonium Sulfate, protect from light 10.0g

Distilled Water 100.0ml

Stir until dissolved. Protect solution from light. 5% Iron

#### **Alum (Ferric Ammonium Sulfate)**

Ferric Ammonium Sulfate , protect from light 5.0g

Distilled Water 100.0ml

Stir until dissolved. Protect solution from light.

#### **Eriochrome (Solochrome) Cyanine Solution**

Eriochrome (Solochrome)Cyanine RS 0.2g

H<sub>2</sub>SO<sub>4</sub> concentratd 0.5ml

Distilled water 96.0ml

10% Iron Alum 4ml

Add H<sub>2</sub>SO<sub>4</sub> to Eriochrome Cyanine in a glass flask.

Add distilled water and stir until dye goes into solution.

Add 10% Iron Alum. Mix well.

Filter into screw--top bottle.

Solution keeps well. Protect from light.

#### **Borax--Ferricyanide Differentiator**

Borax 1.0g

Potassium ferricyanide, protect from light 1.25ml

Distilled water 100.0ml

Stir until dissolved. Protect solution from light.

## **EC Staining Protocol**

- 1.**Take the slides out of the freezer and allow to air dry for a couple hours or overnight at room temperature, or dry the slides on a slide warmer for 1-2 hours at 37c.
- 2.**Place slides in fresh Acetone for 5 minutes.
- 3.**Remove slides from Acetone and let dry for about 10-20 minutes.
- 4.**Place the slides in Eriochrome Cyanine Solution for 30 minutes at room temperature.  
**Note:** The solution was reusable, filter the Eriochrome Cyanine Solution if needed.
- 5.**Rinse the slides with running water.
- 6.**Differentiate in 5% Iron Alum for 5-15 minutes until start to see the gray matter.  
(Usually about 2-5 min for rat spinal cord and 5-10 min for mouse tissue. Do not reuse this solution.)
- 7.**Rinse with running tap water.
- 8.**Complete the differentiation in the borax-ferricyanide solution (reusable) for 5-10 minutes.
- 9.**Rinse with running tap water.
- 10.**Dehydrate briefly through graded ethanol solutions 2 changes each, 70%, 95% and 100%.)
- 11.**Clear briefly through 3 changes of Histoclear.
- 12.**Coverslip using a permanent mounting medium.

### **Results:**

**Myelin:** blue

**Nuclei:** cream/white

**Background:** cream/white

**Note that gray matter and the spinal cord contusion site contain myelin profiles.**