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
H2SO4 concentratrd 0.5ml
Distilled water 96.0ml
10% Iron Alum 4ml
Add H2SO4 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.