**Thionin Protocol For Nissl**

**Description:** Thionin is is a strongly metachromatic dye, useful for the staining of acid mucopolysaccharides. It is a common nuclear stain and can be used for the demonstration of Nissl substance in nerve cells of the CNS.

**Fixative:** 4% paraformaldehyde or 10% neutral buffered formalin

**Sectioning:** 6µm paraffin wax sections

**Solutions:**

LiCO3 solution: Mix 1.65 g of Lithium carbonate into 300 ml of distilled water until dissolved. This will yield 0.55 % LiCO3.

0.1% thionin solution: Obtain 25 ml of this stock solution and dilute with 225 ml of distilled water thus yielding a 0.05% LiCO3. Add 62.5 g of thionin into the solution of 0.05% LiCO3 and stir until dissolved.

**Protocol:**

1. Dewax in Xylene – 2 mins
2. Dewax in Xylene – 2 mins
3. Dewax in Xylene – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Wash in 90% Alcohol – 2 mins
7. Wash in 70% Alcohol – 2 mins
8. Wash in Running Water – 2 mins
9. Treat with LiCO3 for 5 min
10. Stain in Thionin solution for 5 -10 minutes
11. Rinse in distilled water
12. Several Dips in 70 % EtOH, careful this will bleach the stain
13. Leave in 95% alcohol until most of the stain has been removed (butyl alcohol can be used to slow the bleaching)
14. Rapid wash in EtOH 10 dips
15. Clear in Xylene – 2 mins
16. Clear in Xylene – 2 mins
17. Clear in Xylene – 2 mins
18. Mount slides with coverslips using DePeX

**Results:**

Nissl substance ------------------Purple/dark blue

Neurones and cell nuclei ------ Purple/blue