**Nissl Staining Method and Protocol on**

**Paraffin Sections for Brain & Spinal Cord**

 **Description:**This method is used for the detection of Nissl body in the cytoplasm of neurons on paraformaldehyde or formalin-fixed, paraffin embedded tissue sections. The Nissl body will be stained purple-blue. This stain is commonly used for identifying the basic neuronal structure in brain and spinal cord tissue.

**Fixation:**4% paraformaldehyde in 0.1M PBS or 10% formalin.

**Section:**paraffin sections at 5~30 um

**Solutions and Reagents:**

0.1% Cresyl violet solution:

      Cresyl echt violet (or cresyl violet acetate) --- 0.1 g

      Distilled water ------------------------------------ 100 ml

      Add 10 drops (or 0.3 ml) of glacial acetic acid just before use and filter.

**Procedure:**

1. Dewax in Xylene – 10 mins
2. Dewax in Xylene – 10 mins
3. Dewax in Xylene – 10 mins
4. Wash in Absolute Alcohol – 5 mins
5. Wash in Absolute Alcohol – 5 mins
6. Wash in 90% Alcohol – 3 mins
7. Wash in 70% Alcohol – 3 mins
8. Wash in Running Water – 2 mins
9. Rinse in tap water and then in distilled water.
10. Stain in 0.1% cresyl violet solution for 3-10 minutes.

**Note:** Staining in warmed cresyl violet solution (warm up in 37-50 ºC oven) can improve penetration and enhance even staining. It is particularly beneficial for thicker (20-50 um) sections.

1. Rinse quickly in distilled water.
2. Differentiate in 95% ethyl alcohol for 2-30 minutes and check microscopically for best result.
3. Wash in Absolute Alcohol – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Clear in Xylene – 5 mins
7. Clear in Xylene – 5 mins
8. Clear in Xylene – 5 mins
9. Mount slides with coverslips using DePeX

**Results:**

      Neuron (Nissl body) -------------------------- blue-violet