Nissl Staining Method and Protocol on Paraffin Sections for Brain & Spinal Cord

NovaUltra Special Stain Kits

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 PB 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. Deparaffinize sections in xylene 2 or 3 changes at 10 minutes each. Hydrate in 100% alcohol 2x5 minutes, 95% alcohol 3 minutes, 70% 3 minutes. Rinse in tap water and then in distilled water. **Notes:** For 5-10 um sections, 2 changes of xylene, 10 minutes each, will be sufficient. However, for 20-50 um sections, 3 changes of xylene at 10 minutes each, is recommended. **Insufficient deparaffinization will cause uneven staining.**
2. Stain in 0.1% cresyl violet solution for 3-10 minutes. **Notes:** 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.
3. Rinse quickly in distilled water.
4. Differentiate in 95% ethyl alcohol for 2-30 minutes and check microscopically for best result.
5. Dehydrate in 100% alcohol 2x5 min.
6. Clear in xylene 2x5 min.
7. Mount with permanent mounting medium.

Results:

Neuron (Nissl body) ---------- pink-violet

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Positive Controls:

Brain tissue.