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## Nissl Staining Solution (Thionine Method)

**N1507843**

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**Storage:** Room Temperature. Store in the dark.

### Introduction:

Nissl bodies, also known as Nissl granules, are triangular or oval small masses distributed in the cytoplasm of nerve cells. They can be stained purple-blue by basic dyes such as thionine, methylene blue, toluidine blue, and cresyl violet. All types of nerve cells contain Nissl bodies, but their shape, quantity, and distribution location often vary. Nissl bodies are also present in dendrites, but not in axons or the axon hillock of the cell body. Nissl bodies change with the physiological state of the neuron and serve as an important site for protein synthesis within the neuron. When a neuron is stimulated, the number of Nissl bodies in the cell body decreases significantly.

The Nissl Staining Solution (Thionine Method) uses imported thionine acetate as the core dye. It can be used for staining Nissl substances, neurons, and other structures in paraffin tissue sections. The presence or disappearance of Nissl bodies is a key indicator of whether nerve cells are damaged. In cases such as encephalitis, cerebral ischemia, and axonal reaction, Nissl bodies may undergo dissolution or even disappear. The main advantages of the Nissl Staining Solution (Thionine Method) include simple operation, stable staining results, and a wide range of applications. This reagent is for research use only and not intended for clinical diagnosis or other purposes.

### Self-Prepared Materials:

1. series of ethanol solutions, distilled water, Carnoy's fixative or 10% neutral formalin solution, environment-friendly dewaxing solution, neutral balsam, environment-friendly clearing solution, OCT embedding medium, and gradient sucrose solutions.
2. Constant temperature incubator, alcohol lamp, and microscope.

### Operating Steps (for reference only):

1. For paraffin sections: Fix fresh tissues in ethanol, Carnoy's fixative, or 10% neutral formalin solution, followed by conventional dehydration and embedding. Cut sections to a thickness of 6-8  $\mu\text{m}$ , then perform conventional dewaxing with xylene or environment-friendly dewaxing solution until the sections are water-wettable. Rinse with distilled water.
2. For frozen sections: Fix fresh tissues in neutral formalin solution, then dehydrate them in gradient sucrose solutions at 4°C for 24-72 hours until the tissues sink to the bottom.

Embed the tissues in OCT medium and cut into sections with a recommended thickness of 10-15  $\mu\text{m}$ . Immerse the frozen sections in distilled water to rewarm for 3 minutes.

3. Preheat the Nissl Staining Solution (Thionine Method) in an incubator at 50-60°C. When steam appears, immerse the sections in the staining solution and stain for 30-60 minutes.

Rinse briefly with distilled water to remove excess staining solution.

4. Rapidly differentiate the sections with 95% ethanol until the background is nearly colorless. If differentiation fails, repeat Step 3.
5. Dehydrate the sections with absolute ethanol, clear them with xylene or environment-friendly clearing solution, and mount them with neutral balsam.

### **Staining Results:**

Nissl bodies: Purple-blue to dark blue.

Cell nuclei: Light blue.

Background: Colorless or light blue.

### **Precautions:**

1. Nissl bodies are prone to dissolution after the tissue is removed from the body. Therefore, the tissue must be fixed immediately after collection; otherwise, effective staining will be difficult.
2. Tissue fixation plays a crucial role. Ethanol, Carnoy's fixative, or neutral formalin solution can be used for fixation.
3. This staining solution yields better results for Nissl staining of paraffin tissue sections.
4. Differentiation with 95% ethanol should be performed quickly. Observe the sections with the naked eye: the optimal differentiation effect is achieved when the sections are clear and the background is light blue or colorless.
5. Stained specimens must be stored away from light; otherwise, they may fade easily.
6. For your safety and health, wear a lab coat and disposable gloves during operation.
7. Use the reagent as soon as possible after opening to avoid affecting the results of subsequent experiments.