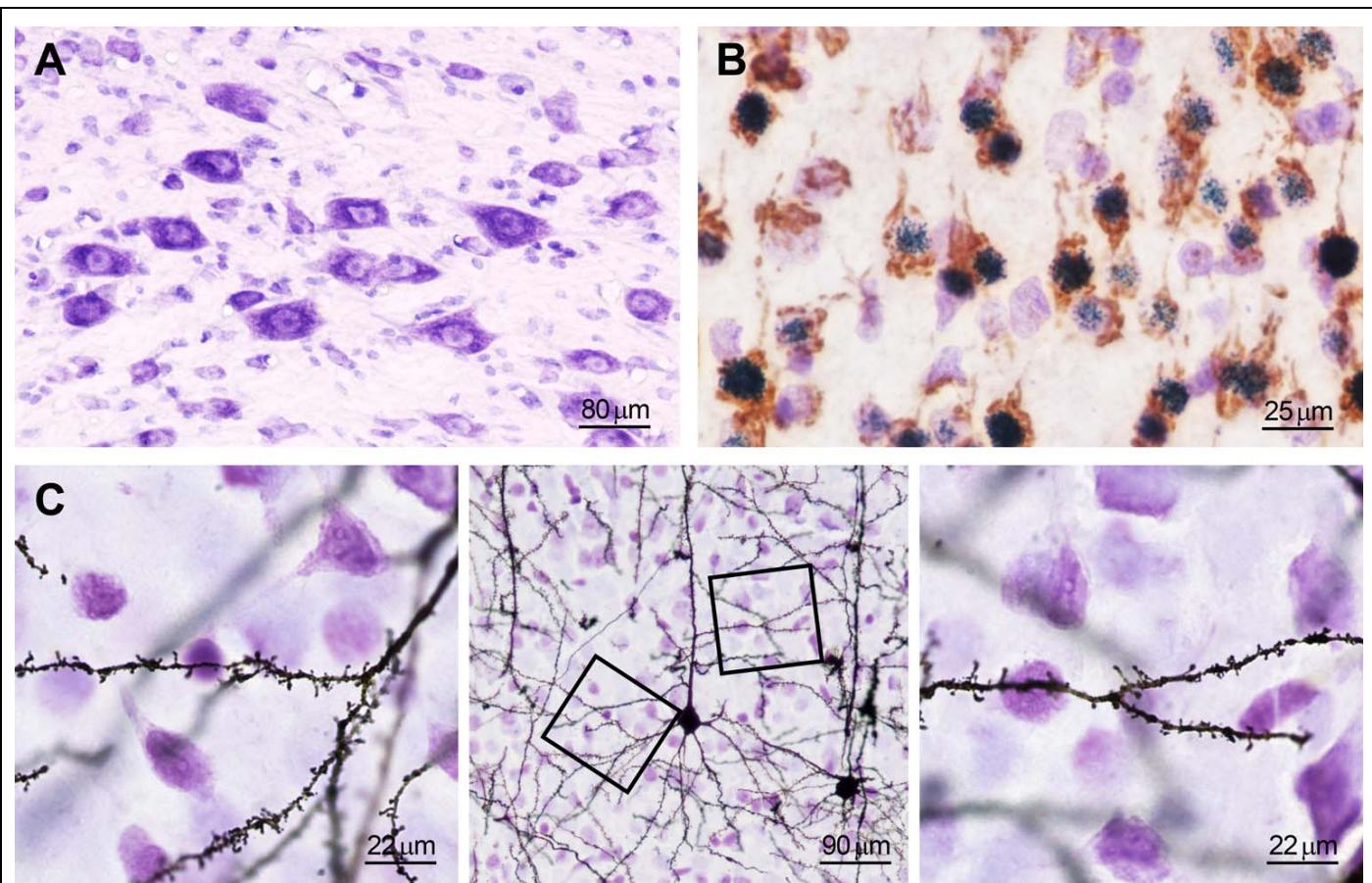


**Cresyl Violet Counterstain Solution**

Laboratory Use Only, Store at 2-25 °C

The Cresyl Violet Solution is formulated for staining Nissl body in the cytoplasm of neurons (Nissl Staining). The Nissl substance (rough endoplasmic reticulum) will be stained purple-blue. This solution has been proven to generate excellent staining on paraformaldehyde fixed sections with or without immunohistochemistry performed, and can be used to identify the basic neuronal structure in brain or spinal cord tissue. The staining can be carried out at room temperature (18-25°C) and generally takes 1-5 minutes. Staining time should be optimized for each tissue type and staining intensity desired. This solution is suitable for use with non-aqueous mountants.

**Cresyl Violet Solution used together with Bioenno DAB/BDHC Substrate Kits and *super*Golgi Kit**

(A) Cryostat section (20 μm thick) stained with the Cresyl Violet solution. (B) The DAB and BDHC Kits were employed to present dual-labelling (brown and blue, respectively), followed by Cresyl Violet counterstaining. (C) Bioenno *super*Golgi Kit was employed to stain the dendritic spines (sections are 150 μm thick), followed by Cresyl Violet counterstaining. Frames in the middle panel were magnified in the left and right panels. All the images were taken from adult mouse brains.

**Warranty:** 12 months from the date of purchase.

**Return Policy:** Bioenno Tech's return policy for this product is 90 days from the date of purchase.

**Free Technical Support:** Email your questions to [contact@bioenno.com](mailto:contact@bioenno.com)

**REAGENT PROVIDED:**

- **Cresyl Violet:** 250 ml of solution in a plastic bottle. The solution is formulated for staining Nissl body in the cytoplasm of neurons. The Nissl substance (rough endoplasmic reticulum) will be stained purple-blue.

**INSTRUCTIONS FOR USE:****(A) Sections with completed immunohistochemistry (IHC/ICC), *in situ* hybridization (ISH), or Golgi staining:**

1. Finish the IHC/ICC, ISH, or Golgi staining, and mount the tissue sections upon adhesive microscope slides. Air-dry the slides at room temperature (RT, 18-25°C).
2. Wash the dry slides in 0.01 M PBS-T for 1-5 minutes and then rinse in dH<sub>2</sub>O for 1-3 seconds.

3. **Individual Slide Staining:** Place the slides on a level surface and apply the solution to sections. Make sure the sections are fully covered with the solution. Incubate the sections at RT for 1-5 minutes depending on the desired intensity and type of tissues. After incubation, wash out extra counterstain solutions with dH<sub>2</sub>O.

**Batch Staining:** Put the Cresyl Violet Solution in a staining jar, and add slides and incubate at RT for 1-5 minutes. After incubation, remove slides and rinse off excess stain solution with dH<sub>2</sub>O.

- Check the stained sections under microscope for the best results.
- Optimal time should be determined by the investigators. Longer time of incubation may be required for some specific tissues.
- The Solution can be reused. If necessary, filter the solution with 0.2-0.4 µm syringe type filter or Whatman<sup>TM</sup> filter paper before use. To enhance the staining intensity, the solution can be warmed up to 40~45°C before the staining.

4. If needed, differentiate the sections in acetic ethanol (70% ethanol containing 0.02% acetate acid) or 70% ethanol for seconds to minutes, and check microscopically for best result. Wash out the ethanol with dH<sub>2</sub>O.
  - The staining intensity of both cellular elements and background decreases quickly in the above acetic ethanol or 70% ethanol.
  - If staining is light, simply reapply the counterstain solution and incubate the sections again.
5. Air-dry the slides and then directly dehydrate sections in 100% ethanol for 1-2 times, 3-5 minutes each. Clear in xylene or xylene substitute, 2-3 changes, 3-5 minutes each. Cover slip with Permount<sup>®</sup> mounting medium.
  - Longer time of Dehydration and Clear may be required for thick Golgi-stained sections.

**(B) Routine histological sections:**

1. Sections should be mounted on gelatin coated or positive charged plus slides. The paraffin-embedded sections should be dewaxed before running the staining.
2. Air-dry the sections/slides. Wash the slides in 0.01 M PBS-T for 1-5 minutes and then rinse in dH<sub>2</sub>O for 1-3 seconds.
3. Stain in the counterstain solution for 1-5 minutes as described above.
4. If needed, differentiate in acetic ethanol or 70% ethanol for seconds to minutes and check microscopically for best result.
5. Dehydrate in 100% ethanol, clear in xylene or xylene substitute, and cover slip with non-aqueous mountants as described above.

**STORAGE, SAFETY, AND HANDLING PRECAUTIONS:**

Store the solution at 2-25°C and avoid strong direct light.

The solution is designed for *in vitro* research use only and not for drug, diagnostic or other uses.

The solution contains reagents that may be harmful in contact with skin, by inhalation or ingestion. Do not pipette by mouth. Use ordinary precautions to avoid inhalation and contact with skin and eyes. In case of contact, wash immediately with generous amounts of water and seek medical advice. If swallowed, wash out mouth with water and immediately call a physician.

Perform experiment under a chemical hood. Wear suitable protective clothing, gloves and eye/face protection. Wash hands thoroughly after performing the experiment.

Material safety data sheet (MSDS) is available upon request.