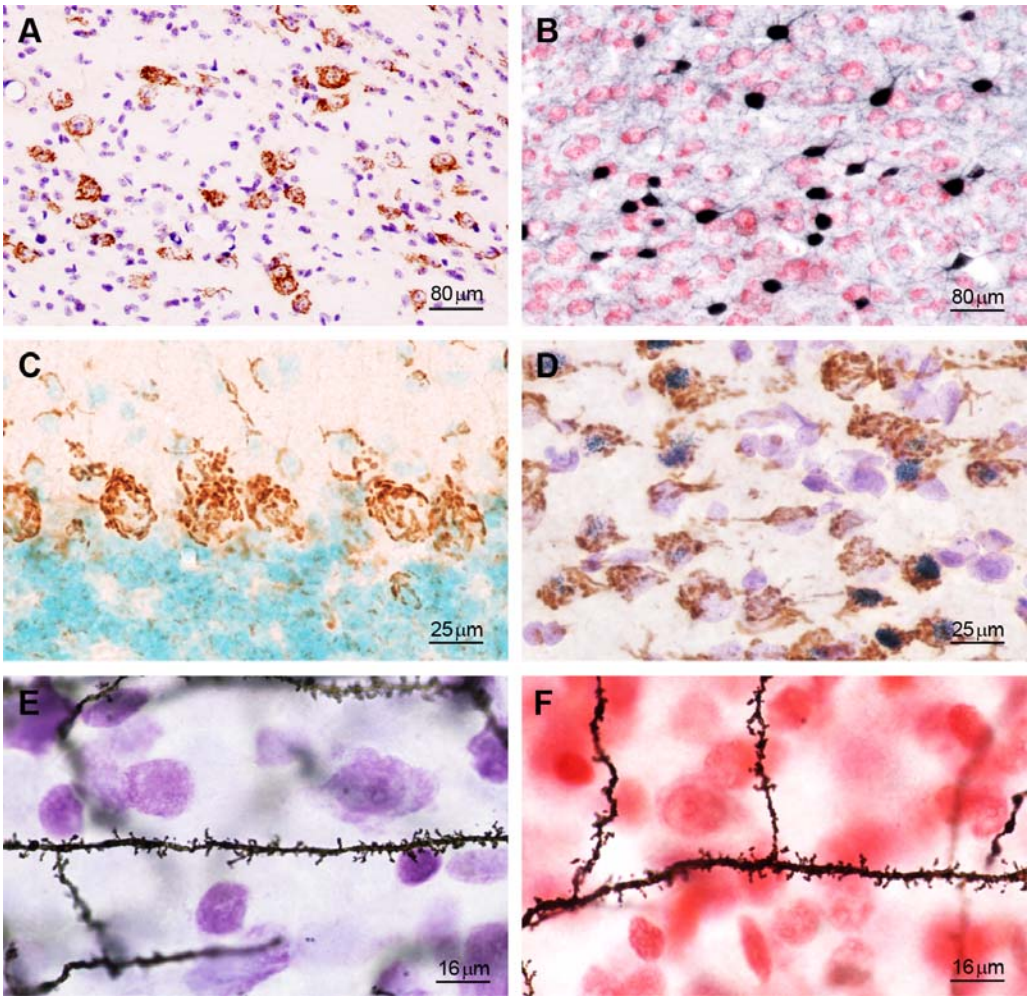
Bioenno Tech, LLC **Counterstain Kit** **Catalog Number:** 003102

Cresyl Violet, Methyl Green, and Neutral Red Solutions

Laboratory Use Only, Store at 2-25 ºC

The Counterstain Kit is specifically designed for running counterstaining on brain sections that have finished the enzyme histochemistry, immunohistochemistry, *in situ* hybridization, or Golgi staining. It also works well on routine histological sections, including frozen and paraffin-embedded tissue sections, as well as cultured cells. The kit provides 3 counterstain solutions (red, green, and purple-blue) that are ready to use straight from dropper bottles. The unique formulas of these dye solutions allow researchers to produce the most reliable and specific staining of cellular elements with lower background.



Counterstain Kit used together with Bioenno DAB/BDHC Substrate Kits and *super*Golgi Kit

(A) Cryostat section counterstained with the Cresyl Violet solution. The immunoreactive products (brown) were first developed with Bioenno DAB Kit. (B) DAB-Co Kit was used to develop the immunoreactive neurons (bluish black), followed by Neutral Red counterstaining. (C) Methyl Green counterstaining. The brown Purkinje cells were developed with DAB Kit. (D) Both DAB and BDHC Kits were employed to present dual-labelling (brown and blue, respectively), followed by Cresyl Violet counterstaining. (E,F) Bioenno *super*Golgi Kit was employed to stain the dendritic spines (150 µm thickness of sections), followed by Cresyl Violet (E) and Neutral Red (F) counterstaining. 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***

REAGENTS PROVIDED WITH THE KIT:

- **Cresyl Violet**: 30 ml of solution in a dropper 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.
- **Methyl Green**: 30 ml of solution in a dropper bottle. Methyl green is a nuclear counterstain that stains cell nuclei light green. The solution has been purified with chloroform.
- **Neutral Red**: 30 ml of solution in a dropper bottle. The solution can be used as a red nuclear counterstain in various histological procedures.
- **Acetic Ethanol**: 30 ml of differentiation solution in a dropper bottle. Differentiation of staining is not necessary in most cases. However, incubating sections in this solution may be helpful for eliminating non-specific background staining. The solution contains 70% ethanol and 0.02% acetate acid.

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, 18-25ºC).

2. Wash the dry slides in 0.01 M PBS-T for 1-5 minutes and then rinse in dH_2O for 1-3 seconds.

3. Place the slides on a level surface and apply one of the counterstain solutions 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_2O.
- 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.

4. If needed, differentiate the sections in the kit provided acetic ethanol or 70% ethanol for seconds to minutes, and check microscopically for best result. Wash out the ethanol with dH_2O.
- The staining intensity of both cellular elements and background decreases quickly in the 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® 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_2O for 1-3 seconds.

3. Stain in one of counterstain solutions 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 kit at 2-25ºC and avoid strong direct light.

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

The kit 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 while handling kit reagents. Wash hands thoroughly after performing the experiment.

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