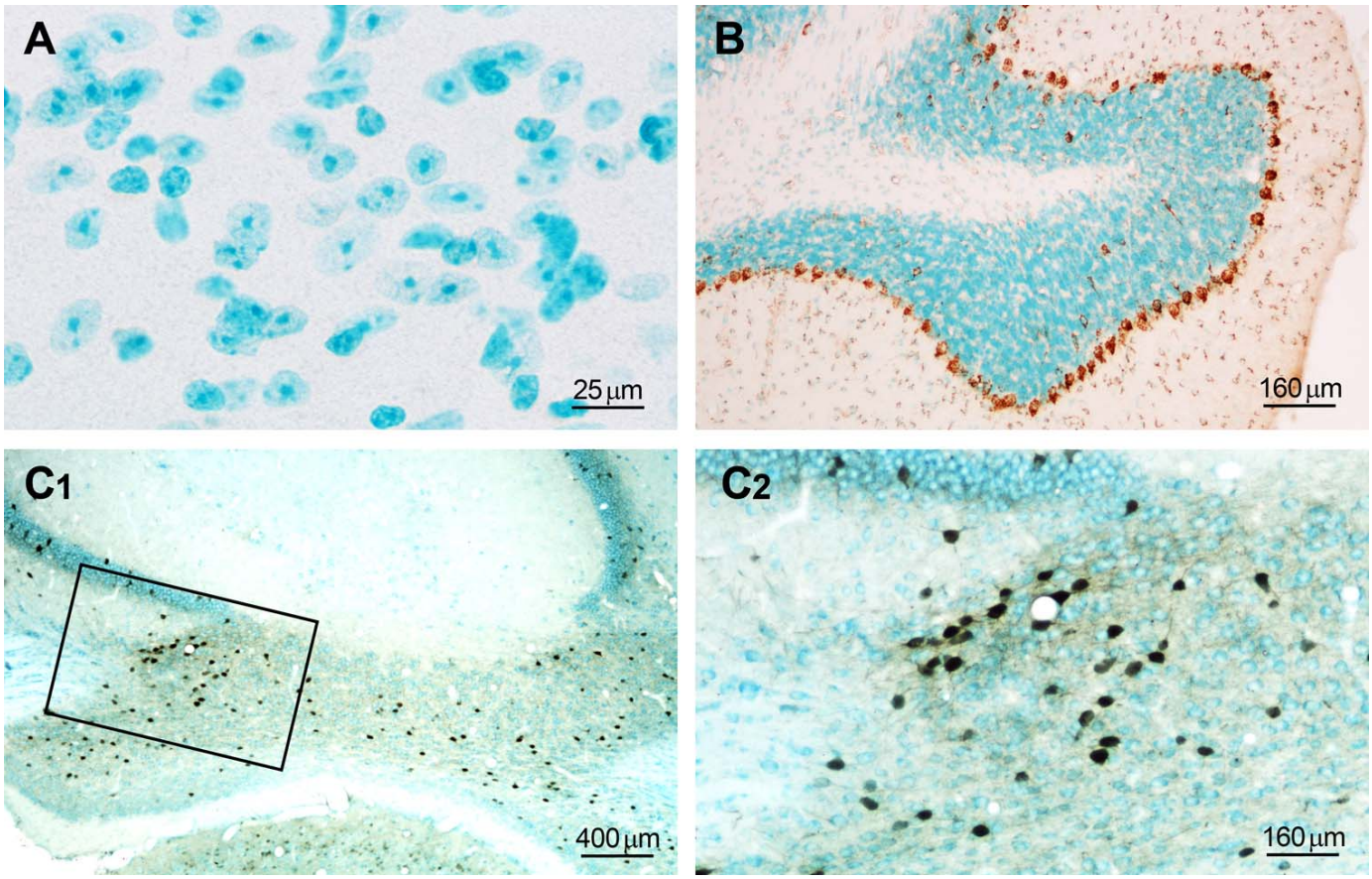
Methyl Green Counterstain Solution

Laboratory Use Only, Store at 2-25 ºC

Methyl Green is a nuclear counterstain that stains cell nuclei light green. The Bioenno Methyl Green Solution can be applied to routine histological sections, sections with completed histochemistry, immunohistochemistry, *in situ* hybridization, or Golgi staining, as well as cultured cells. The Bioenno formulated solution has been purified with chloroform and has proven to generate excellent nuclei staining on immunohistochemistry sections developed with 3,3'-diaminobenzidine (DAB) or benzidine dihydrochloride (BDHC). The staining can be carried out at room temperature (18-25ºC) and generally takes 1-5 minutes. Differentiation is not necessary in most cases. This counterstain solution is suitable for use with non-aqueous mountants.



Methyl Green Solution used together with Bioenno DAB and DAB-Co Substrate Kits

(A) Cryostat section stained with the Methyl Green solution. (B) Bioenno DAB Substrate Kit was first employed to develop the immunoreactive products (brown), followed by Methyl Green counterstaining. (C) DAB-Co Kit was used to develop the immunoreactive neurons (bluish black), followed by Methyl Green counterstaining. All the images were taken from adult mouse brains. The cryostat sections are 20 µm thick.

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***

REAGENT PROVIDED:

- **Methyl Green**: 250 ml of solution in a plastic bottle. Methyl green is a nuclear counterstain that stains cell nuclei light green. The solution has been purified with chloroform.

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. **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_2O.

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_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.
- The Solution can be reused. If necessary, filter the solution with 0.2-0.4 µm syringe type filter or Whatman™ filter paper before use. To enhance the staining intensity, the solution can be warmed up to 40~45ºC 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_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 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 2-25ºC 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.