

CRESYL VIOLET STAIN

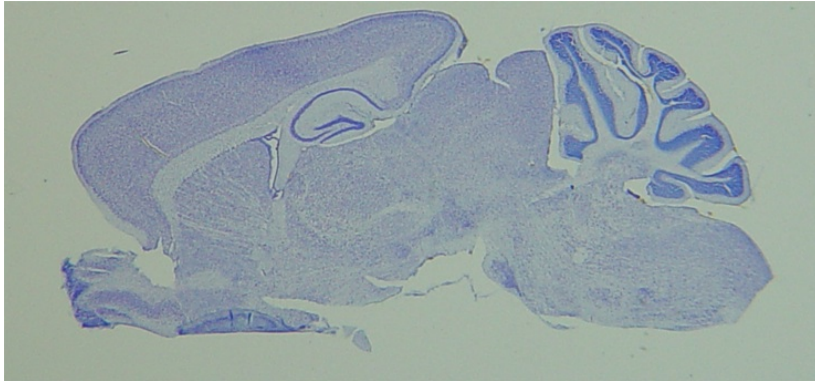


Figure 1. Sagittal view of the mouse brain stained with cresyl violet. Cresyl violet stains the somas of neurons fixed with formalin by binding to nissl substance.

Overview.

Nissl substance is found in fixed neurons and includes the granular endoplasmic reticulum and ribosomes occurring in the soma and dendrites. Also called *nissl body* and *nissl granule*. Nissl substance is visible only in fixed tissues after staining with basic aniline dyes (see: Humason, 1983; Deitch and Moses, 1957).

The method described here is for 20-50 μm thick formalin (3 to 10%) fixed frozen brain sections. This method will also work for paraffin embedded tissues with several additional steps to remove the imbedding media (provided at the end of this protocol). The stain will identify the somas of neurons (a violet-purple color), but not glial cells (see Figures 1 to 4). This stain is often used when you need to count the number of cells (e.g., following administration of a neurotoxin), or if you need to measure the size of lesions or find an electrode placement. The stain is probably the easiest and most reliable stain for neurons and is one of the most widely used stains in brain research.

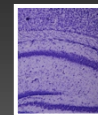
References

Deitch, A. D. and Moses, M. J. (1957). The nissl substance of living and fixed spinal ganglion cells. *The Journal of Biophysical and Biochemical Cytology*, **3(23)**: 449-456.

Humason, G. L. (1983). *Animal Tissue Techniques*, San Francisco, W. H. Freeman and Company.

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Applications

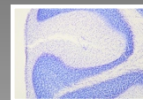


Anatomical studies in where the goal is to study the distribution and location of neurons.

Experiments requiring the verification of lesion size and location.

Experiments requiring the verification of the placement of cannula implants or electrodes.

Limitations



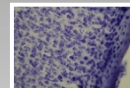
Staining is limited to the soma of neurons.

Glia are left unstained.

Neuron processes are unstained.

Tissues must be fixed in formalin.

Slide Characteristics



Slides are stable in light and can last for decades with very little care.

Slides are easily viewed and photographed with a light microscope at 4x to 60x.



Required Materials

11 staining wells (see above) or for small runs of 8 or less slides Coplin jars will work.

1 or more slide holders

Lab oven or incubator preheated to 60 C (140 F)

Cresyl violet acetate (CAS 10510-54-0 for example: Sigma chemical # C0542)

Reagent grade alcohol 600 ml each of 70, 95 and 100% concentrations (can be reused for several weeks)

Magnetic stir plate and stir bars

Sodium acetate

Acetic acid

Distilled water (600 ml per run – be sure to replace after each run).

xylene based mounting media (e.g., Cytoseal 60)

coverslips

prepared subbed slides with tissues sectioned at 20 to 50 μ m warmed to room temperature just before use (20 minutes is enough if slides were stored after sectioning in the refrigerator).

Mixing Cresyl violet for 300 ml staining wells

1.) Cresyl violet Stock solution:

0.2 g cresyl violet-acetate in 150 ml distilled water. Mix with a stir bar for at least 20 minutes. Note: this stir bar will be purple forever so use a dedicated bar for this purpose (on the “bright” side you will always know which one to use as it will be the purple one).

2.) Buffer solution pH 3.5:

a.) 282 ml of 0.1 M acetic acid (6 ml of concentrated acetic acid per 1000 ml distilled water)

and add to

b.) 18 ml of 0.1 M sodium acetate (13.6 g in 1000 ml of distilled water)

3.) **Final step:** Now take 30 ml of the cresyl violet stock solution and add 300 ml of buffer. Mix for at least 30 minutes. This stain is stable for at least six months depending on the number of slides. If staining is light just increase the amount of time in the oven up to 16 minutes.

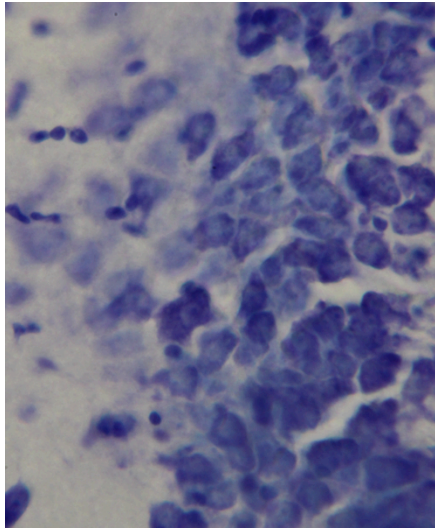


Figure 3. Mouse brain sectioned at 40 μ m and stained for 10 minutes (60x magnification).

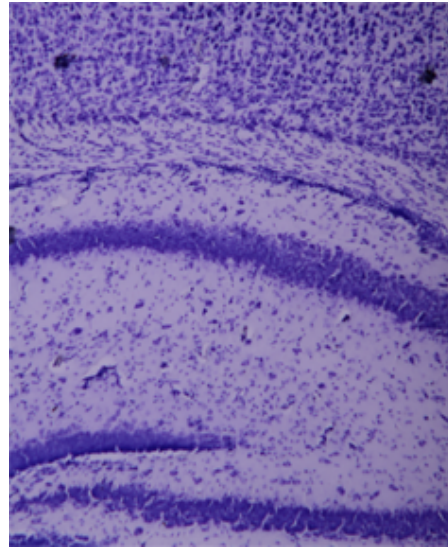


Figure 4. Mouse brain sectioned at 40 μ m and stained for 8 minutes (10x magnification).

Staining Procedure for Frozen Sections

Put the well for step 5 (containing the stain) in an oven or incubator for at least an hour at 60 C prior to staining. But, wait to set up your other wells until about 20 minutes before you start. Use a hood for all steps other than step 5 (oven/incubator).

Sectioned tissues mounted on slides can be stored for months in the refrigerator (4 C). Load slides into holders (make sure they all face the same way) twenty minutes before staining to allow them to warm to room temperature. Then put the holder into the following wells containing the following solutions for the times indicated.

Steps

- 1.) Xylene (5 minutes)
- 2.) 95% Alcohol (3 minutes)
- 3.) 70% Alcohol (3 minutes)
- 4.) Deionized distilled water for 3 minutes
- 5.) Cresyl Violet (8-14 minutes depending on the age of the stain and the species) at 60 C (oven)
- 6.) Distilled water (3 minutes)
- 7.) 70% Alcohol (3 minutes)
- 8.) 95% Alcohol (1-2 minutes)
- 9.) 100% Alcohol (up to 10 dips to remove any streaks; there are rarely any streaks to remove so 1 dip is probably enough). Be very careful at this step or you will remove all of your stain and have to start over.
- 10.) Xylene (5 minutes)
- 11.) Place in next Xylene well and keep lid closed. The slides can stay in this well until coverslipped. But the longer they are in the more likely some will fall off. Up to 24 hours is usually OK. Use a Xylene based mounting media and top grade coverslips. For fewest air bubbles and best long-term slide storage the slides should be in steps 10 and 11 for a combined total of at least 30 minutes.

Technical Notes:

- 1.) HistoClear was used in steps 1, 13 and 14 until 1997 when it was no longer produced. We tried Americlear and Sigma Xylene substitute but we found that the stain did not penetrate the cells adequately. So xylene (which Americlear replaced) is still at this time the best clearing agent. We use reagent grade chemicals from either Sigma or Carolina Biological and obtain very good results.
- 2.) The important variables seem to be the freshness of the differentiation solutions and the temperature of the cresyl violet solution during staining (60 C).
- 3.) **IMPORTANT:** Tissues must be mounted on subbed slides or they will fall off during staining. To make subbed slides, dissolve 1 g of gelatin in 1 liter of hot distilled water. Cool and add 0.1 g of chromium potassium sulfate. Store in refrigerator. Dip slides 4 times drying the slides in a hood at room temperature between dips. This is Humason's (1983) modification of Boyed (1955). The finished slides can be stored in a covered slide box at room temperature indefinitely.
- 4.) Step 5: Mouse, rat, chick and iguana require 8 minutes to stain. Human and sheep require 14 minutes. If you obtain sheep brains fixed in Carosafe then you must block the tissues into 1 cm blocks or smaller and fix them in 3% formalin for at least two weeks prior to sectioning.
- 5.) There is more than one type of cresyl violet stain. Make sure you buy cresyl violet acetate.
- 6.) After sectioning, unstained slides should be stored in the refrigerator until stained. There does not appear to be a time limit for storing slides.
- 7.) This stain only works in formalin fixed tissues. We use a 3% PBS buffered formalin fixation for at least two weeks (no upper limit). It is not necessary to perfuse smaller brains (e.g., mouse, rat, chick, rabbit) as formalin penetrates 1 cm very quickly. Brains where the distance from the exterior surface to the center of the brain is > 1 cm must either be perfused or blocked to 1 cm or less to ensure proper fixation without shrinking. Older protocols call for fixation at 10%. This is not necessary and newer pollution control standards require close monitoring of formalin so reducing the concentration of formalin below 10% is strongly recommended.

Additional Steps to remove embedding media (occurs before step 1 of the staining procedure).

- 1.) xylene 5 minutes
- 2.) xylene 5 minutes
- 3.) proceed with step 1 of the staining protocol

You can stain with paraffin in place but the staining time in step 5 must be extended (up to an hour or longer) and you need to add an additional 10 minute xylene step at the end before coverslipping (in addition to the recommended 35 minutes in steps 10 and 11).

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