BLACK GOLD II STAIN FOR MYELIN

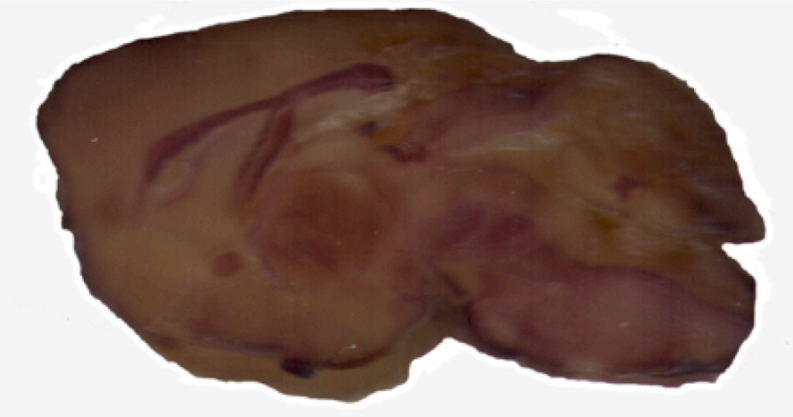


Figure 1. Sagittal view of the mouse brain stained using the gold chloride method of Wahlsten, Colbourne, and Pleus, 2003: J. Neuroscience Methods **123**: 207-214). Myelin appears red to reddish brown.

Overview.

Various gold chloride methods have been developed to stain myelin but with minimal success as the stain general only penetrates a mm or two into the tissue section (see Figure 1). These methods have been mainly used in studies to verify the presence of the corpus callosum which does stain very well using this method. But the method involves staining a large block (usually an entire hemisphere) to visualize only midline structures. The method does not adapt to staining thin sections.

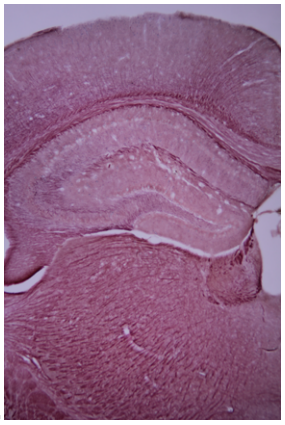
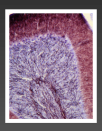


Figure 2. Mouse brain stained with Black Gold II 40 μm thick section.

The recent development of Black gold II as a myelin stain allows for the staining of sections 20 to 50 μm thick (see Figure 2). The result is a stable and vibrant slide that shows myelin in any part of the brain. So far we have been successful staining formalin fixed chick, mouse, rat, sheep and human brain with consistent results. The method does not work with fresh tissue. The method also allows for counter staining with cresyl violet.

Version 3-9-13

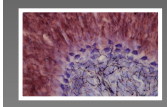
Applications



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

Experiments requiring the verification and size of the corpus callosum.

Limitations



Staining is limited to the myelin of neurons. No other white matter is stained.

Tissues must be fixed in formalin for 10 to 30 days (no longer).

Tissue from young subjects does not appear to stain.

Slide Characteristics

Slides are stable in light. Storage time is not known but we have stored slides for at least three years.

Slides are easily viewed and photographed with a light microscope at 4x to 40x.

Required Materials

18 Coplin jars.
Plastic forceps
Lab oven or incubator preheated to 60 C (140 F)
Black Gold II (www.histo-chem.com 150 mg (Product # 1BGII)
Cresyl violet acetate (CAS 10510-54-0 for example: Sigma chemical # C0542) **(optional only if counterstaining)**
Reagent grade alcohol 150 ml each of 70, 95 and 100% concentrations (can be reused for several weeks)
Magnetic stir plate and stir bars
Sodium acetate **(optional only if counterstaining)**
Sodium Thiosulfate
Acetic acid **(optional only if counterstaining)**
Distilled water (1080 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 µm warmed to room temperature just before use (20 minutes is enough if slides were stored after sectioning in the refrigerator).

Black Gold solution

Vortex all powders before opening to knock material from inside of caps. Resuspend Black Gold II powder in 0.9% saline to a final concentration of 0.3%

Add 150 mg of Black Gold II powder into 50 mL of 0.9% NaCl (9 g/L) mix to resuspend. Store in amber bottle at 4 C for up to 3 months.

Note: this is only enough to partially fill a Coplin jar if you use 5 slides and the sections do not reach more than an 1" closer to the end of the slide. If you have 10 slides it will be enough to cover the sections.

Sodium thiosulfate (1%) mix just before use.

Cresyl violet (optional only if counterstaining)

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.

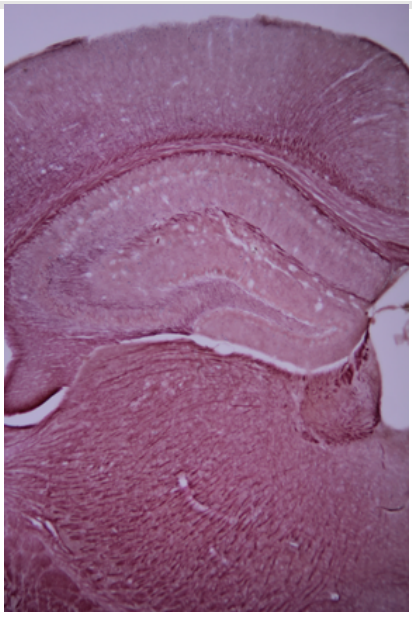


Figure 3. Mouse brain sectioned at 40 µm and stained with Black Gold II alone (4x magnification).

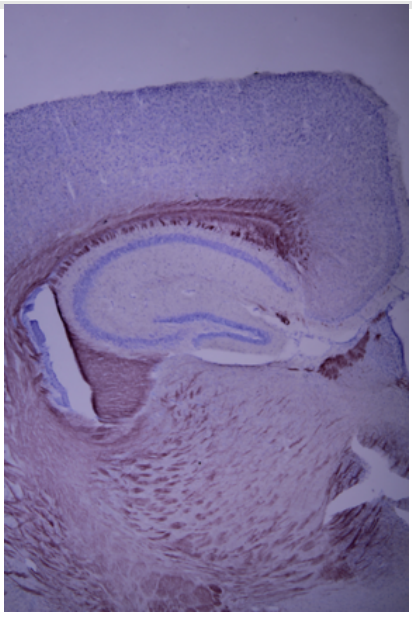


Figure 4. Mouse brain stained with Black Gold II (red to black) and counterstained with cresyl violet (purple). The slide is shown at 4x magnification. Notice that in this mouse strain the corpus callosum does not cross to the other hemisphere.

Staining Procedure for Formalin Fixed Frozen Sections

Important: Preheat 0.3% Black Gold II and 1% sodium thiosulfate solutions to 60 C (for about an hour). Use a hood for all steps requiring xylene.

Sectioned tissues mounted on slides can be stored in the refrigerator (4 C). Load slides two per slot (back to back) into the Coplin jar for step 1 (make sure they all face tissue side out) twenty minutes before staining to allow them to warm to room temperature. Then move the slides using a plastic forceps into the following wells containing the following solutions for the times indicated.

Steps

1.) Hydrate sections in distilled water for 2 minutes
2.) Incubate slides in Black-Gold II solution at 60 C for 12 minutes
3.) After 12 minutes; monitor the slides at 2-3 minute intervals and stop incubation when the finest myelinated fibers are staining dark red to black. The appearance of a lavender background color indicates over staining and the process should be stopped. If the desired color has not appeared by 30 minutes its time to change to a fresh solution.
4.) Rinse in distilled water 2 minutes.
5.) Rinse in distilled water 2 minutes.
6.) Stain in sodium thiosulfate (1%) at 60 C for 3 minutes.
7.) Rinse in distilled water 2 minutes.
8.) Rinse in distilled water 2 minutes.
9.) Rinse in distilled water 2 minutes.
10.) Stain in cresyl violet at RT or in the oven at 60 C for 3 minutes.
11.) Rinse in distilled water 2 minutes.
12.) Rinse in distilled water 2 minutes.
13.) Rinse in distilled water 2 minutes.
14.) dehydrate in 70% ETOH for 3 minutes
15.) 95% ETOH for 3 minutes
16.) 100% ETOH for 3 minutes
17.) xylene for 30 minutes or longer. May be left overnight in xylene if covered tightly in a fume hood. You will want multiple xylene jars to hold the finished slides until you are ready to coverslip.
18.) coverslip

Technical Notes:

The important variables seem to be the duration of fixation prior to staining, freshness of the Black Gold II solution, and the temperature of the stain and sodium thiosulfate during staining 60 C).

1.) Staining at below 60 C is very poor and requires significantly more time.

2.) As you stain more slides the amount of time required in the stain will increase. In the first run staining will be complete in 12-15 minutes. The staining time will increase each time you use the stain. Depending on the species we can usually stain 50 to 60 slides (250 to 300 sections) before the stain no longer works and we routinely get much more out of each batch (150 mg of stain). The appearance of a black residue at the bottom of the staining jar does not seem to matter nor is it a good indicator that the stain has run out. When staining takes more than 35-45 minutes it is usually time to mix new stain. Reusing the stain over several months has not been a problem as long as it is stored in the refrigerator.

3.) This stain only works in formalin fixed tissues (nonembedded). Fixation should generally be for at least a week but less than a month. Mouse and rat brains really don't stain well if fixed longer than a month (staining becomes fainter the longer the tissue is fixed). But some of the best slides we ever made were from a chick brain fixed for 10 years! We have also found that brains from mice less than 10 days old do not stain at all. We use a 3% PBS buffered formalin fixation for two weeks. 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.

4.) **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.

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.

Wahlsten, D., Colbourne, F., and Pleus, R. (2003). A robust, efficient and flexible method for staining myelinated axons in blocks of brain tissue. *Journal of Neuroscience Methods* **123**: 207-214).