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BLACK-GOLD II MYELIN STAINING KIT

CATALOG NUMBER: AG105 **QUANTITY:** 1 kit

LOT NUMBER:

DESCRIPTION: Black-Gold II is an aurohalophosphate complex which stains specifically for myelin within the central nervous system. Black-Gold II staining provides high resolution, high contrast, a short histochemical processing time, and high reproducibility. Black-Gold II is a new and improved version of its predecessor, Black-Gold (Cat # AG390). The advantages of Millipore's Black-Gold II Myelin Staining Kit over Black Gold I are that it is more readily soluble, can be utilized at a higher concentration, produces a more uniform and consistent staining, takes less time to stain, and does not require post-staining intensification procedures.

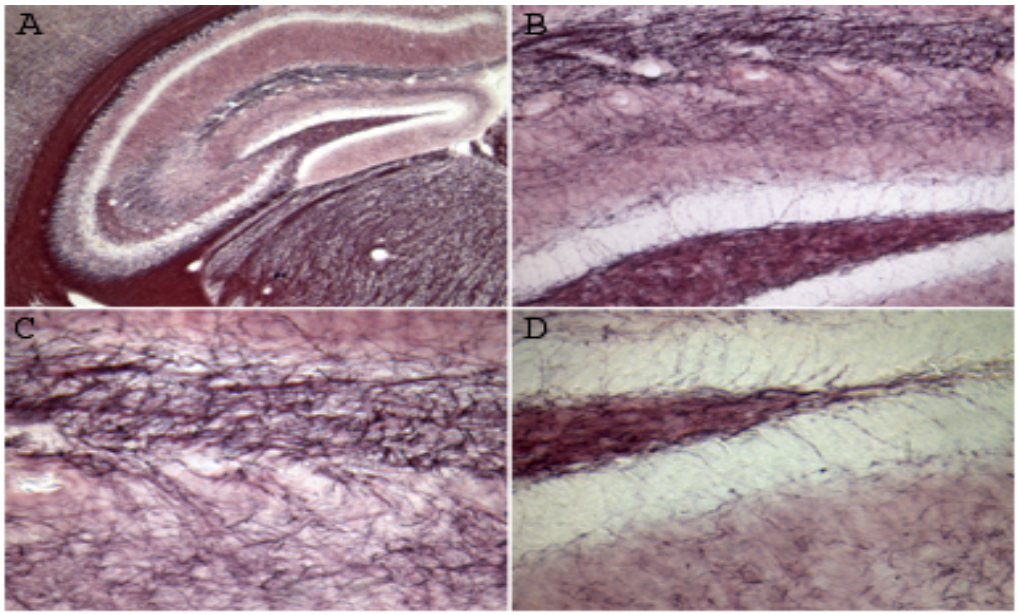

Figure 1. Examples of Black-Gold II staining of cryosectioned mouse brain tissue. A) Low power magnification (5X) of hippocampus, thalamus, and part of the sensory motor cortex. B) 20X view of the hippocampus. C) High magnification image (40X) of the molecular layer. D) 40X view of the dentate gyrus. Take note how large bundles of myelin have a deep red appearance whereas individual fibers appear nearly black.

The use of Black-Gold II is tailored to studies using formalin-fixed, non-solvent processed tissue. The technique stains large myelinated tracts dark red-brown, while the individual myelinated fibers appear black. This novel tracer can be used to localize both normal and pathological myelin. Black-Gold II can demonstrate and characterize specific myelin changes associated with exposure to diverse neurotoxicants including kainic acid, methamphetamine, acrylamide, domoic acid, 3-nitropropionic acid, Fluoro-Gold and isoniazid.

The Black-Gold II Myelin Staining Kit also comes with sodium thiosulfate and cresyl violet. The sodium thiosulfate solution is required for extracting excess Black-Gold II stain. Whereas the cresyl violet is a Nissl stain that colors cell bodies a brilliant bluish hue and is used as a counter stain with Black-Gold II.

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Black Gold II Purity:	No detectable amount of uncomplexed gold was found.
Black Gold II Illumination:	Bright field or dark field.
Black Gold II Solubility:	Freely soluble in water, saline, or dilute acids.

COMPONENTS:

Black-Gold II: 150mg dry powder, yellow in color.
Cresyl Violet Stain: 150mg dry powder, purplish-black in color.
5% Sodium Thiosulfate: 10ml

STORAGE/HANDLING:

The kit can be stored at room temperature. Ideally, the Black-Gold II powder should be kept in a desiccator, because of its hygroscopic nature. The dry powder, if unopened and stored properly, is useable for up to one year from date of receipt. Once resuspended the Black-Gold II staining solution should be stored in the dark at 4℃ and is good for up to 3 months.

TOXICITY: Although the compound appears to be of low toxicity, it has not been extensively evaluated and therefore routine laboratory caution should be exercised. Not intended for human consumption.

PROTOCOL FOR USE:

Tissue Fixation:
Tissue can be fixed with either formalin or 4% PFA. Typically animals are perfused with neutral phosphate buffered 10% formalin (or 4% formaldehyde) via the ascending aorta. The brains are then removed and fixed overnight in the same fixative solution. Longer fixation times are generally not recommended. For cryosections, the brains may be protected through treatment with sucrose solution (20-30 percent). Subsequently frozen brain tissue is cut on a freezing sliding microtome. Note: Black-Gold II will not stain paraffin embedded or unfixed tissue.

Sectioning:
Either frozen or fixed, non-frozen vibratome sections can be used and should be cut at a thickness of 20-40µm. The sections are then typically mounted on positively charged slides. The sections can also be stained loose, although the sections are easier to handle when mounted on slides.

Solutions:
Black-Gold II powder should be resuspended with saline solution (0.9% NaCl) to a final concentration of 0.3%. Simply add the 150mg of Black-Gold II powder into 50mls of 0.9% NaCl solution and mix to resuspend. Once resuspended the Black-Gold II staining solution should be stored in the dark at 4℃ and is good for up to 3 months.
Cresyl Violet powder should be resuspended to a final concentration of 0.1% using a mixture of MilliQ water and glacial acetic acid (3µl of acid per ml of water). Once resuspended the cresyl violet solution should be stored at 4℃ and is good for up to 6 months.
The sodium thiosulfate should be diluted to 1% using MilliQ water prior to use.

Staining:
Pre-heat 0.3% Black-Gold II and 1% sodium thiosulfate solutions to 60℃. Rehydrate tissue sections in MilliQ water for 2 minutes then transfer to pre-warmed Black-Gold II solution. Incubate slides in Black-Gold II solution at 60℃ for 12 minutes. At this point the slides should be monitored at 2-3 minute intervals to determine the extent of labeling. Staining is complete 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. *Note:* The exact staining time will vary depending on section thickness, fixing conditions, type of

tissue, and age of stain solution.

The sections are then rinsed 2X in MilliQ water for 2 minutes each. Sodium thiosulfate solution (1%) is then added and incubated for 3 minutes at 60℃. The sections are then rinsed 3X with MilliQ water for 2 minutes each. Cresyl violet solution is then added and incubated for 3 minutes at room temperature. The sections are then rinsed 3X with MilliQ water for 2 minutes each.

Post-staining steps:
Finally, dehydrate the slides using a series of gradated alcohols. The cresyl stain will leach from the tissue at this time and the extent of staining can be controlled by how long these incubations last. The dehydrated sections are then cleared in histoclear or xylene for 2 minutes and then coverslipped with mounting media.

Frequently Asked Questions (FAQs):

1) It appears that Myelin impregnation is incomplete with Black-Gold II?
Answer: Return slides to 60℃ Black-Gold II solution and monitor every 2-3 minutes under microscope until fine parallel fibers of layer 1 cortex are visible.

2) Background has lavender color?
Answer: Section has been overstained and staining time should be reduced.

3) Background staining occurs before impregnation of all fine myelinated fibers?
Answer: Tissue was probably over-fixed. Try to avoid leaving brain in fixative, or sections in buffer, for longer than one month.

4) What are the advantages over other myelin stains like Luxol fast blue, or Sudan Black?
Answer: Better resolution and contrast. Faster.

5) Do other counter stains work with Black-Gold II?
Answer: Yes, most conventional Nissl stains (including blue, red, and green) can be used as a counterstain. Additionally, the counter stain can be omitted if only myelin staining is desired.

REFERENCES:

Schmued, L et al. (2008). Introducing Black-Gold II, a highly soluble gold phosphate complex with several unique advantages for the histochemical localization of myelin. Brain Res. 1229: 210-217.

Bowyer, J et al. (2008). Neurotoxic-related changes in tyrosine hydroxylase, microglia, myelin, and the blood-brain barrier in the caudate-putamen from acute methamphetamine exposure. Synapse. 62(3): 193-204.

Schmued, L. and Slikker, W. (1999). Black-gold: a simple, high-resolution histochemical label for normal and pathological myelin in brain tissue sections. Brain Res. 837: 289-297.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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