

BLACK-GOLD®

(a novel marker for normal and pathological myelin)

CATALOG NUMBER:	AG390	QUANTITY:	100 mg
LOT NUMBER:			
DESCRIPTION:	<p>Black-Gold® is a novel haloaurophosphate complex which localizes myelin within the central nervous system. The use of Black-Gold® is tailored to studies using formalin-fixed, non-solvent processed tissue. The technique stains large myelinated tracts dark red-brown, while the individual myelinated axons appear black. This novel tracer can be used to localize both normal and pathological myelin. Black-Gold® can demonstrate and characterize specific myelin changes associated with exposure to diverse neurotoxicants including kainic acid, domoic acid, 3-nitropropionic acid, Fluoro-Gold and isoniazid. Black-Gold® can also be combined with other histochemical markers including Nissl stains, retrogradely transported fluorescent tracers and fluorescent markers of neuronal degeneration. The advantages associated with the Black-Gold® include high resolution, high contrast, short histochemical processing time, versatility, and consistent reproducibility.</p> <p>APPEARANCE: Mahogany brown colored powder.</p> <p>PURITY: No detectable amount of uncomplexed gold was found.</p> <p>ILLUMINATION: Bright field or dark field.</p> <p>SOLUBILITY: Soluble in warm (e.g. 60°C) water or saline.</p>		
PRESENTATION:	Dry powder, brown in color.		
STORAGE/HANDLING:	<p>Store powder at room temperature in a well sealed vial, preferably in a desiccator, because of its hygroscopic nature. The 0.2% Black-Gold® staining solution in saline (0.9%) should be stored in the dark. The staining solution may be reused for up to 2 months depending on amount of tissue processed. The dry powder, if unopened and stored properly is useable up to a year from date of receipt.</p> <p>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.</p>		
USAGE:	<p><u>SUGGESTED PROTOCOL FOR USING BLACK-GOLD®</u></p> <p>Fixation: Tissue can be fixed with either 4% PFA or formalin. The use of unfixed (fresh) frozen or fixed, paraffin embedded tissue is not recommended. Typically animals are perfused with 500 mL of 0.1 M neutral phosphate buffered 10% formalin (4% formaldehyde) via the ascending aorta. Then the brains are post-fixed overnight in the same fixative solution. Longer fixation times are not recommended (see FAQ #3). For cyrosections, the brains may be protected through treatment with twenty percent sucrose. Subsequently frozen brain tissue is cut on a freezing sliding microtome.</p>		

Sectioning:

Either frozen or fixed, non-frozen vibratome sections can be used and should be cut at a thickness of 20-50 μm and collected in 0.1 M neutral phosphate buffer. The sections are then typically mounted on slides and then air dried on a slide warmer (at 50°C) for at least half an hour. The sections can also be stained loose, although the sections are easier to handle when mounted on slides.

Staining:

The mounted sections are rehydrated in distilled water for 2 minutes before transferred to a warm Black-Gold® solution. A 0.2% solution of Black-Gold® should be made by adding 100 mg of Black-Gold® to 50 mL of 0.9% NaCl and then heating it to 60°C. To do this, the solution is typically heated in a microwave oven to the approximate temperature, and then allowed to fully equilibrate to 60°C in a conventional oven. The solution does not usually need to be filtered, but it can be if precipitates form after long use, or pieces of tissue are present (upon reuse usually). The slide mounted tissue sections are transferred into this warm Black-Gold® impregnating solution in the oven for 12-18 minutes. *Note:* The exact staining time will vary depending on section thickness and solution temperature. It is advisable to initially monitor the staining visually. The sections are typically examined after 12 minutes.

Staining of the fine parallel fibers of the molecular layer of the cortex is a good indicator of proper impregnation. If the section is under-impregnated these fibers will not be visible, indicating that the section should be placed in the staining solution again and microscopically examined at 2 or 3 minutes intervals. If the section is left in the staining solution too long, it will become over impregnated allowing the neuropil in the molecular layer to acquire a lavender background color. At this point, it is possible to intensify the stain by incubating the sections for 10-15 minutes in a 60°C solution of 0.2% potassium tertachloroaurate (Alrich Chem., Milwaukee, WI) dissolved in 0.9% saline. The intensified or non-intensified sections are then rinsed for 2 minutes in distilled water, fixed for 3 minutes in a 1% sodium thiosulfate solution, and then rinsed in tap water for at least 15 minutes (three 5 minute changes). *Note:* When stored in the dark, both impregnation and intensification solutions typically remain stable and useable for at least 2 months.

Post-staining steps:

Finally, the slides can be either air-dried on a slide warmer or dehydrated through graded alcohols. The dehydrated sections are cleared in xylenes (Fisher Scientific, Pittsburgh, PA) for at least 2 minutes and then coverslipped with D.P.X. (Fluka Chem Group, Ronkonkoma, NY) plastic mounting media. Other mounting media can be used if the technique is not to be combined with fluorescent microscopy.

Frequently Asked Questions (FAQs):

- 1) It appears that Myelin impregnation is incomplete with Black-Gold®?
Answer: Return slides to 60°C Black-Gold® solution and monitor every 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.



REFERENCES:

- Eickhoff, S et al. (2004). High-resolution MRI reflects myeloarchitecture and cytoarchitecture of human cerebral cortex. *Hum Brain Mapping* 24(3):206-215.
- Harrist, A. et al. (2004). Alteration of hippocampal cell proliferation in mice lacking the beta2 subunit of the neuronal nicotinic acetylcholine receptor. *Synapse* 54(4):200-206.
- Dieni, S et al. (2004). The Pattern of Cerebral Injury in a Primate Model of Preterm Birth and Neonatal Intensive Care. *J Neuropath Exp Neurol* 63(12):1297-1309.
- Chem, Ming-Kai et al (2004). Peripheral benzodiazepine receptor imaging in CNS demyelination: functional implications of anatomical and cellular localization. *Brain* 127(6):1379-1392.
- Prinz, M et al. (2004). Intrinsic Resistance of Oligodendrocytes to Prion Infection. *Neurobiol Dis* 24(26):5974-5981.
- Walters, NB et al. (2003). In vivo identification of human cortical areas using high-resolution MRI: An approach to cerebral structure–function correlation. *PNAS, USA* 100(5):2981-2986.
- Lipska, BK et al. (2002). Effects of Reversible Inactivation of the Neonatal Ventral Hippocampus on Behavior in the Adult Rat. *J Neurosci* 22(7):2835-2842.
- Wang, L et al. (2002). Liver X receptors in the central nervous system: From lipid homeostasis to neuronal degeneration. *PNAS, USA* 99(21):13878-13883.
- Garman, RH et al. (2001). Methods to Identify and Characterize Developmental Neurotoxicity for Human Health Risk Assessment. II: Neuropathology. *Environ Health Pers Suppl* 109(S1).
- Hopkins, KJ et al. (2000). Temporal progression of kainic acid induced neuronal and myelin degeneration in the rat forebrain.. *Brain Res* 864(1):69-80.
- 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. {original article}.

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 μ L 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.*

For research use only; not for use as a diagnostic.

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