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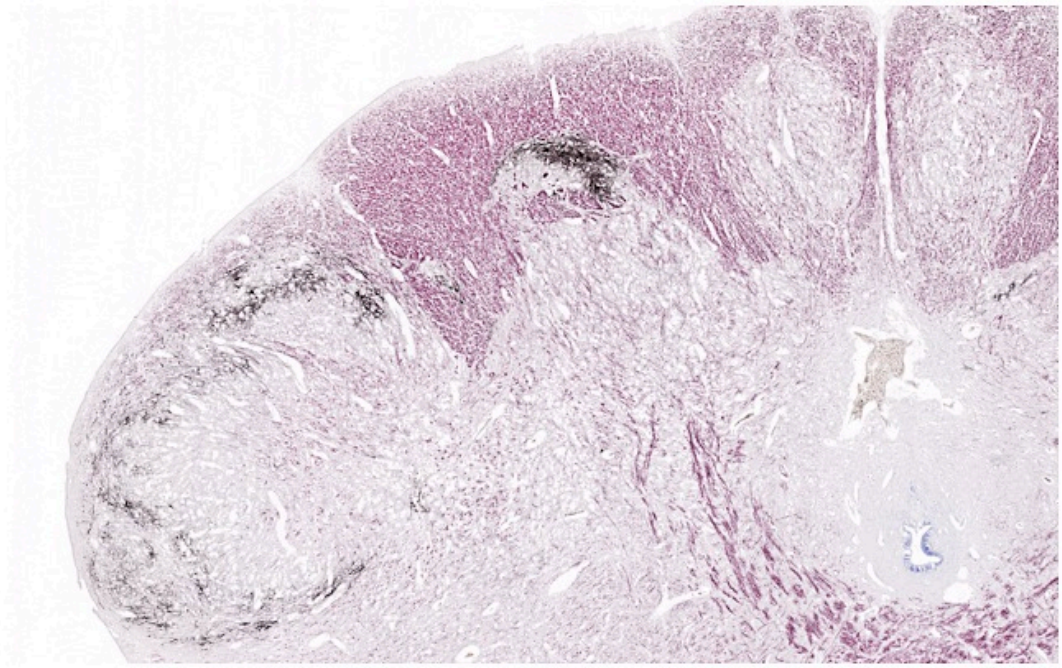
Black-Gold® II RTD™ Myelin Stain Reagent with Toluidine Blue O Counter Stain Protocol

Catalog Number: TR-100-BG



For research use only, not for use in clinical and diagnostic procedures.

1. Background Black-Gold II:

Black-Gold® II is a novel chemical auro-halophosphate complex compound that has a particular affinity for myelin and can be used to localize myelin within the central nervous system. 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. Additionally, Black-Gold® II can be combined with other histochemical markers including Nissl stains, retrogradely transported fluorescent tracers and fluorescent markers of neuronal degeneration. Biosensis is proud to introduce a ready-to-dilute (RTD) version of the Black-Gold® II designed to make the use and research with this novel compound easier and more consistent.

2. Description

The Biosensis Black-Gold® II RTD™ kit is a high-resolution myelin stain with Nissl counter stain for use on 10% formalin-fixed, **non-embedded brain tissues**, including 4% PFA fixed, frozen tissues. The alcohol pretreatments necessary for paraffin embedding or with acetone/ethanol fixed material are incompatible with Black-Gold II® staining, unfortunately.

The Toluidine Blue O or Nissl counterstain is a classic neuronal stain which identifies Nissl bodies (endoplasmic reticulum) and nuclei as the dye will stain primarily DNA and RNA. The staining method is useful to localize the perikaryon, or cell body, and dendrites of neurons, but not the axon or axon hillock. Because the nucleic acids are basophilic they are stained blue in a classic Nissl stain.

3. Materials Provided

- 10X Black-Gold II, Solution A (10 mL)
- 10X Sodium Thiosulfate, fixative, Solution B (10 mL)
- 10X Toluidine Blue O, Solution C (10 mL)
- 10X Acetic Acid, Solution D (10 mL)

4. Storage Conditions

The kit can be stored unopened for up to 6 months at 2-8ºC after date of receipt. We recommend storage of kit components protected from light. After opening the kit the components can be stored up to an additional 3 months at 2-8ºC. Diluted solutions can be stored up to one month at 2-8ºC. We recommend using aseptic techniques when handling the reagents to avoid contamination.

5. Materials Required but not Supplied

- Gelatin-coated tissue slides
- Staining dishes/Coplin jars
- Cover slips
- DPX mounting media
- Slide warmer
- Convection oven or water bath
- Distilled water
- Ethanol
- Xylene
- Bright field or dark field illumination microscope of camera system

6. Preparation of Gelatin-Coated Slides

The slides are prepared by placing clean slides in a slide rack and placing in a solution of ethanol for 2 minutes, then placing in distilled water for 2 minutes. The slides are then transferred to a 1% pig-skin gelatin solution, (Sigma: 300 Bloom) which has been heated to 65°C. Drain excess gelatin on paper towel and transfer to paraffin-free convection oven overnight at 60°C. After overnight drying the slides are ready to be used to mount fresh-cut formalin-fixed sections.

Note: Commerically prepared slides can also be used. Our gelatin-coated slides provide an easy protocol for those that want to make their own slides that have maximum adhesion capabilities. Black-Gold® II has successfully been used with SuperFrost Gold slides as well as other makes of charged slides. For best results it is essential that cut sections be thoroughly dried on to any slide for best adhesion.

7. Myelin Staining Protocol

1. Fixed tissue sections are first mounted from distilled water onto gelatin-coated slides and then air dried at 50-60°C for at least 30 minutes on a slide warmer until thoroughly dry.
2. Slides with tissue sections are then rehydrated in distilled water for about 2 minutes. Do not over-hydrate or tissues can come off slides, prepare only as many hydrated slides as needed for one examination.
3. To a clean beaker or Coplin jar, add 9 parts distilled water to 1 part staining Solution A (Black-Gold® II) and heat to 65°C in a convection oven or water bath; microwaving is not recommended as it can cause precipitation of the dye. Incubate slides for about ~12 minutes in the dark, 60-65°C, covered to prevent evaporation. Microscopic monitoring of the extent of the labeling is recommended. This monitoring should be repeated every 2-3 minutes until the desired degree of myelin impregnation is observed (see below).
4. Rinse the slides for about 2 minutes in distilled water by placing them into a clean Coplin jar or beaker full of distilled water of 50-100 mL volume
5. In a clean Coplin jar or beaker, add 1 part Solution B (sodium thiosulfate, fixative) to 9 parts distilled water and place rinsed slides into it; allow the slides to incubate for 3 minutes.
6. Rinse the slides with either 3 x 5 minutes changes of tap water or 15 minutes of running tap water. Proceed to step 8 if counter stain is not desired.
7. OPTIONAL NISSL COUNTER STAIN: Immerse sections in 70% ethanol for 2 minutes. Transfer slide to a solution of 1 part Solution C (Toluidine Blue O) to 9 parts of 50% ethanol for 5 minutes. The sections are then rinsed in 50% ethanol for 1 minute. The differentiating solution is prepared by adding 1 part Solution D (acetic acid) with 9 parts of 70% ethanol with the slide immersed for 30 seconds. The slides are then transferred through 2 x 2 minute submersions in 100% ethanol. Proceed to step 9 below.

8. Dehydrate sections either via graduated alcoholic solutions or by air-drying on a slide warmer.
9. Immerse sections for 1-2 minutes in xylene and then coverslip with a non-aqueous (i.e. non-polar) mounting media such as DPX or Permount. Other mounting media can be used if the technique is not to be combined with fluorescent microscopy.

Variations, Modifications and Additional Procedures

This high-contrast and -resolution myelin stain is only applicable to tissue that has been formalin-fixed and cannot be used on solvent extracted (eg. paraffin or plastic embedded) tissue.

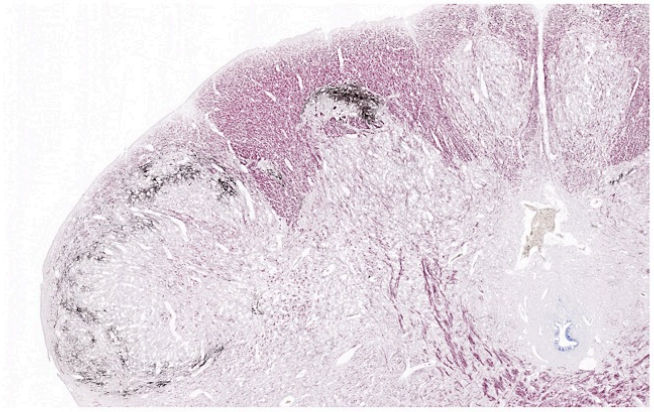
As fixation is critical, both intravascular perfusion and immersion post-fixation is recommended. Intervals typically range from 1-7 days prior to sectioning. Excessively long (eg., 1 year or more post-fixative) storage in formaldehyde may result in the loss of impregnation of the finest myelinated fibers. Fixative may consist of 10% formalin or 4% paraformaldhyde dissolved in either neutral phosphate buffer or physiological saline. Tissue sections can be stored for a few weeks in neutral 0.1M phosphate buffer. For longer storage, sections should be stored below 0°C in an anti-freeze solution such as equal parts glycerin, ethylene glycol and phosphate buffer.

Slide Monitoring: When monitoring the staining, it is complete when the finest myelinated fibers (eg. the parallel fibers in layer 1 of the cortex) are impregnated. The appearance of a conspicuous lavender colored background stain indicates that the tissue is becoming over-stained and should be stained no longer. The exact optimal staining time will vary according to factors such as the temperature and age of the staining solution. The staining solution can still be used even after a fine black precipitate appears at the bottom of the staining dish. However, staining times in excess of 20 minutes suggest that the working solution has lost its strength and should, therefore, be discarded.

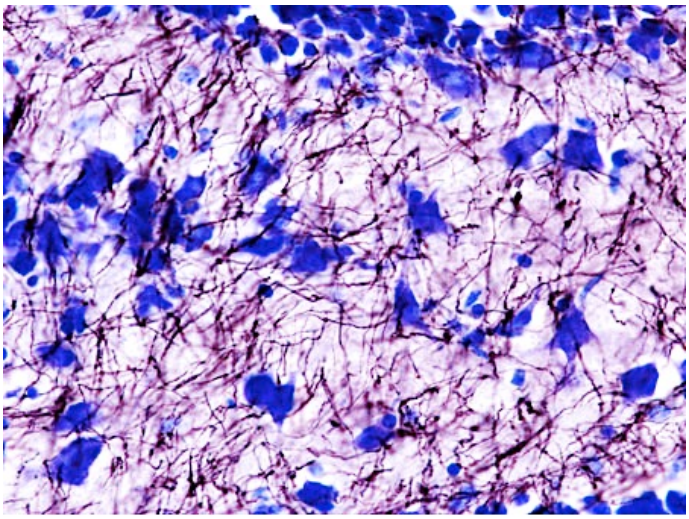
Since the Black-Gold® II staining is highly temperature dependent, it is important to maintain the correct constant temperature. The Black-Gold® II staining solution's temperature should be fully equilibrated before use. Avoid cooling of staining solution when monitoring staining.

Black-Gold® II can be visualized either via bright field or dark field illumination. The Toluidine Blue O Nissl stain can be visualized with bright field illumination. If the Toluidine Blue O Nissl stain has a noticeably high background stain, it can be further differentiated in Solution D (acetic acid in 70% ethanol). If over-differentiated, it can be restained with the dye solution and then differentiated in Solution D for less time.

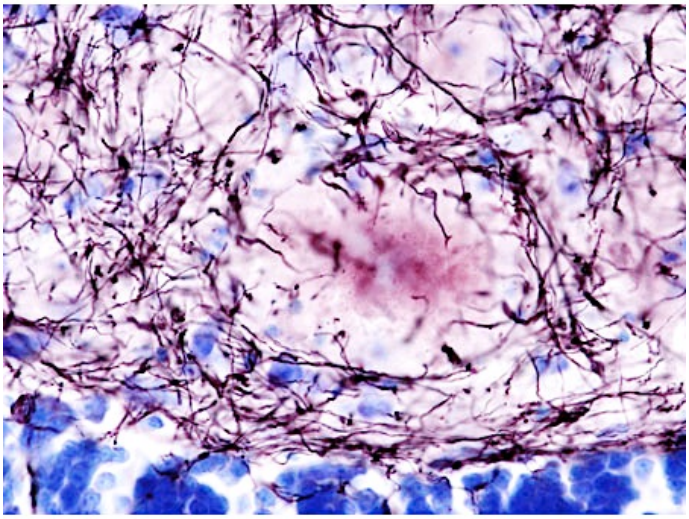
8. Example Images



Calcitonin gene-related peptide-like immunoreactivity (black) and myelin staining (red, Black-Gold II staining kit, Biosensis) in the medulla oblongata, showing the similar neurochemical and structural organization of the dorsal column nuclei Locus K and the spinal trigeminal substantia gelatinosa. Adapted from Del Fiacco M. *et al.* (2018). *Cells* 7(7).



Bright field illumination (60X magnification) of the dentate gyrus of a normal mouse reveals individual Black-Gold II stained myelinated fibres and Toluadine Blue O stained polymorph cells (centre) and granule cells (top). Photo is courtesy of Dr. Larry Schmued.



Bright field illumination (60X magnification) of 8 month old Ad-Tg mouse hippocampus. The myelin pathology can be observed in and around amyloid plaques. The cell bodies of adjacent granule and polymorph cells appear blue, while individual myeliated fibres appear nearly black. Photo is courtesy of Dr. Larry Schmued.

9. Specific References

Please refer to our website for the latest product-specific publications highlighting the use of Black-Gold® II RTD™ Myelin Stain Reagent with Toluidine Blue O Counter Stain.