Black Gold II Staining Kit with Toluidine Blue O Counter Stain

PROTOCOL

Black-Gold II is a high-resolution myelin stain with Nissl counter stain. For use on formalin fixed, non-embedded brain tissue.

EQUIPMENT AND REAGENTS NEEDED

- Staining dishes or Copland jars
- Slide warmer
- Coverslips
- Convection oven or water bath
- Distilled water
- Ethanol
- Xylene
- DPX mounting media

This kit allows the investigator to localize myelin, both individual fibers and tracts, along with the option of co-localizing cell bodies. Black-Gold II labeled myelinated fibers appear nearly black, while Toluidine Blue O labeled cellular Nissl bodies are blue under bright field illumination. Kit is designed so that all stock solutions can be diluted 1:9 with solvent (usually distilled water, occasionally ethanol). No additional reagents needed. 10ml of the stock solutions reconstitute to make a total of 100ml of working solutions.

Instructions

1. Tissue sections are first mounted from distilled water onto gelatin-coated slides and then air dried at 50-60 degrees C for at least ½ hour on a slide warmer. The slides are prepared by placing clean slides in a slide rack and placed in a solution of ethanol for 2 minutes, and then placed 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 degrees C. Drain excess gelatin on a paper towel and transfer to paraffin free convection oven overnight at 60 degree C.
2. Slides with tissue sections are then rehydrated in distilled water for 2 minutes.
3. Add 9 parts distilled water to 1 part staining Solution A (Black-Gold II) and heat to 65 degrees C in a convection oven or water bath. Incubate slides for about 12 minutes. 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 2 minutes in distilled water.
5. Add 1 part Solution B (sodium thiosulfate, fixative) to 9 parts distilled water and allow slides to incubate for 3 minutes.
6. Rinse the slides with either 3 5-minute 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 two minute submersions in 100% ethanol. Proceed to step 9.
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.

Variations, Modifications and Additional Procedures

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

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STAINING KITS

Black Gold II Staining Kit with Toluidine Blue O Counter Stain

Fluoro-Jade C Staining Kit with DAPI Counter Stain

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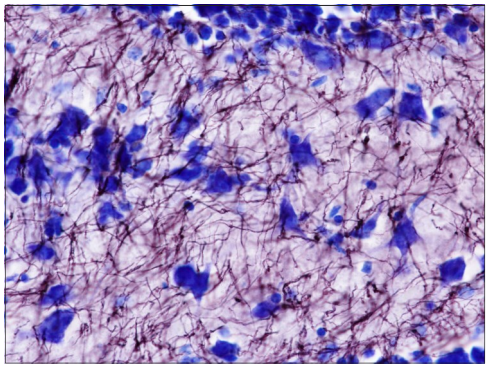
As fixation is critical, both intravascular perfusion and immersion post-fixation is recommended. Intervals typically range from 1-7 days prior to sectioning. Excessively long (eg. 1 year or more) storage in formaldehyde may result in the loss of impregnation of the finest myelinated fibers. Fixative 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 degrees C in an anti-freeze solution such as equal parts glycerin, ethylene glycol and neutral phosphate buffer.

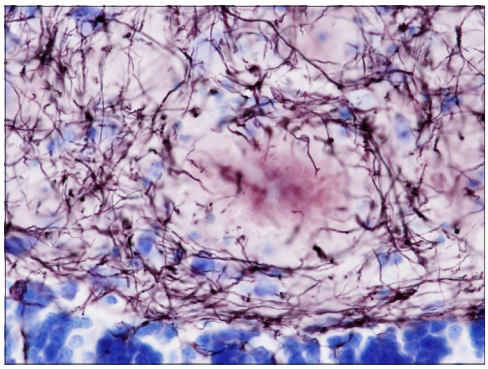
When monitoring the staining, it is complete when the finest myelinated fibers (eg. the parallel fibers in layer 1 of the cortex) are impregnated. The appearance of a conspicuous lavender colored background stain indicates that the tissue is becoming overstained and should be stained no longer. The exact optimal staining time will vary some, 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 via either 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 re-stained with the dye solution and then differentiated in Solution D for less time.



High magnification of the dentate gyrus of a normal mouse reveals individual Black-Gold II stained myelinated fibers and Toluadine Blue O stained polymorph cells (center) and granule cells (top). Bright field illumination, 60X mag.



Comparable field of view of the 8 month old AD-Tg mouse hippocampus. Note myelin pathology in and around amyloid plaques. The cell bodies of adjacent granule and polymorph cells appear blue, while individual myeliated fibers appear nearly black. 60X mag.

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