biosensis® Ready-to-Dilute (RTD)™ Black-Gold® II Staining Kit Protocol

(with Toluidine Blue O counter stain)

Catalog No: TR-100-BG

The biosensis RTD (Ready-to-Dilute) Black-Gold® II kit is a high-resolution myelin stain with Nissl counter stain counter stain for use on 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 ® 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 basophilic they are stained stained blue in a classic Nissl stain.

MATERIALS PROVIDED

Black-Gold II, Solution A (Dilute 1:10 prior to use) - 10 mL
Sodium Thiosulfate, fixative, Solution B (Dilute 1:10 prior to use) - 10 mL
Toluidine Blue O, Solution C (Dilute 1:10 prior to use) - 10 mL
Acetic Acid, Solution D (Dilute 1:10 prior to use) - 10 mL

EQUIPMENT AND REAGENTS NEEDED

Gelatin coated tissue slides
Staining dishes/Copland jars
Cover slips
DPX mounting media
Slide warmer
Convection oven or water bath
Distilled water
Ethanol
Xylene

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.

STORAGE CONDITIONS

The kit can be stored unopened for up to 12 months at 2-8ºC after date of receipt. We recommend that you store the kit components protected from light. After opening the kit the components can be stored up to an additional 6 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.

INSTRUCTIONS

1. 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.
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 Copland 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. 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 Copland jar or beaker full of distilled water 50-100 mL volume
5. In a clean Copland 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 3x5 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 two 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.

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 (e.g. 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.

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 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 restained with the dye solution and then differentiated in Solution D for less time.