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

(with Congo Red counter stain)

Catalog No: TR-200-BGT

The biosensis RTD™ (Ready-to-Dilute) Black-Gold® II Kit is a high-resolution myelin stain with amyloid plaque 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 Congo red stain is a classic neuronal stain for amyloid and it stains amyloid red in bright field illumination and when excited with a green wavelength light, the Congo red stained amyloid will fluoresce orange.

MATERIALS PROVIDED:

Black-Gold II, Solution A (Dilute 1:10 prior to use) - 5 mL
Sodium Thiosulfate, fixative, Solution B (Dilute 1:10 prior to use) - 5 mL
Sodium Hydroxide, Solution C (Dilute 1:10 prior to use) - 5 mL
Congo Red, Solution D (Dilute 1:10 prior to use) - 5 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 minute changes of tap water or 15 minutes of running tap water. Proceed to step 8 if counter stain is not desired.
7. OPTIONAL CONGO RED AMYLOID PLAQUE COUNTER STAIN: Mix in a clean Copland jar, 1 part Solution C (sodium hydroxide) to 9 parts distilled water and incubate slides 5 minutes. Transfer slides to staining solution made by adding 1 part Solution D (Congo Red) to 9 parts 50% ethanol (diluted with water) and incubate slides for 20 minutes. Then rinse slides for 1 minute in an ethanol series consisting of 50% ethanol for 1 minute, then one minute 1 minute in 70% ethanol (this is the differentiation /destaining step) , then finally Immerse slides for 2 minutes in 2 changes of 100% ethanol. Proceed to step 9.
8. Dehydrate sections either via graduated alcoholic solutions (as noted in step seven for staining) or by air-drying until dry on a 37°C 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. Do not use glycerol as it will blur the images.

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 (e.g. 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 (e.g. 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 Congo Red amyloid plaque stain can be visualized either with bright field or epi-fluorescent illumination (via green light excitation). Simultaneous localization of both tracers is best achieved by combining dark field illumination of the Black-Gold® II, with the fluorescent localization of the Congo Red.

If the Congo Red stain for amyloid plaques is overstained, it can be differentiated longer in 70% ethanol. If the plaques are understained, they may be returned to the staining solution and then differentiated in 70% ethanol for less time.