Special Stain Kit
Congo Red, Amyloid Stain

Catalog No: 38016SS9

Intended Use
For In Vitro Diagnostic Use. For Laboratory Use.
The Congo Red, Amyloid Stain Kit when used with appropriate histological protocols may be used for the detection of amyloid deposits in tissue sections.

Summary and Explanation
Amyloid is an amorphous eosinophilic material that is composed primarily of fibrillary proteins arranged in a linear ß pleated sheet orientation. Deposits of amyloid occur in various organs including spleen, liver and kidneys as a consequence of chronic inflammatory diseases. Other diseases associated with amyloid deposits include Multiple Myeloma and Alzheimer’s dementia.

Although the exact mechanism by which Congo Red stains amyloid is unknown, it is generally believed that the Congo Red molecule binds the linearly arranged amyloid fibrils through hydrogen bonds. When viewed with polarized light microscopy, red to green birefringence occurs due to the parallel alignment of the dye molecules on these linearly arranged amyloid fibrils.

The specificity of this technique is enhanced by using a staining solution with an elevated pH and high salt concentration. These conditions reduce the likelihood of non-specific electrostatic interactions of Congo Red with molecules other than amyloid.

Positive staining with Congo Red and the resultant apple green birefringence with cross polarization, is generally considered the most specific method available to the light microscopist for the detection of amyloid.

Reagents Provided
Congo Red Solution (Item No. 38016SS9A, 500 mL)
Potassium Hydroxyxide Solution (Item No. 38016SS9B, 100 mL)
Gill II Hematoxylin (Item No. 38016SSAC, 500 mL)

Please see reverse side for hazardous ingredients and warning symbols.

Storage and Stability
Store at room temperature (15-30 °C). Do not use after the expiration date.

Specimen Preparations

Fixation
Neutral buffered formalin is recommended for fixation. Prolonged storage in formalin based fixatives, may result in a gradual decrease in staining intensity of amyloid deposits. Other fixatives recommended in literature citations include absolute alcohol, Carnoy’s, and Bouin’s fixative.

Paraffin Sections
Following processing and paraffin embedding, section tissue specimens at 6–10 microns. Amyloid deposits in sections less than 6 microns in thickness may fail to demonstrate birefringence.

Preparation of Congo Red Working Solution
Add 0.5 mL of the Potassium Hydroxide Solution to 50 mL of the Congo Red Solution. Mix well, filter and store in tightly capped bottle. The Congo Red working solution is stable for up to 7 days.

(Please note: Exercise caution when handling Potassium Hydroxide Solution.)

Staining Protocol (Conventional, Room Temperature)
1. Deparaffinize tissue sections with xylene and hydrate through graded alcohols to deionized water.a
2. Rinse in 3 changes of deionized water.
3. Place in 95% ethanol for 5-10 seconds.
4. Stain in the Congo Red working solution for 20 minutes.b
5. Rinse in 5-8 changes of deionized water.
6. Stain in Gill II Hematoxylin for 1-3 minutes.
7. Wash in running tap water for 2 minutes.
8. Rinse in 3 changes of deionized water.
9. Dehydrate in two changes of 95% alcohol and three changes of absolute alcohol for one minute each.c
10. Clear in two changes of xylene and mount in a xylene miscible medium.

Staining Protocol (Microwave)
Exercise caution when using the microwave to heat any solution or reagent. The microwave must be properly ventilated to prevent the accumulation of fumes in the laboratory. Microwave transparent Coplin jars and caps should be used during the staining process. The caps should be loosely attached to prevent spills. Caps with ventilation holes also may be used.

All microwaves should be used in accordance with the manufacturer’s instructions. The procedures described here were performed using an Energy Beam Sciences H2250 laboratory microwave. Because of differences in microwave power and frequencies among different models, it may be necessary to adjust power levels or times to achieve optimal results.

1. Deparaffinize tissue sections with xylene and hydrate through graded alcohols to deionized water.a
2. Rinse in 3 changes of deionized water.
3. Place in 95% ethanol for 5-10 seconds.
4. Place sections in a plastic Coplin jar containing the Congo Red working solution and microwave at 500 watts for 30 seconds.
5. Rinse in 5-8 changes of deionized water.
6. Stain in Gill II Hematoxylin for 1-3 minutes.
7. Wash in running tap water for 2 minutes
8. Rinse in 3 changes of deionized water.
9. Dehydrate in two changes of 95% alcohol and three changes of absolute alcohol for one minute each.c
10. Clear in two changes of xylene and mount in a xylene miscible medium.

Technical Notes
a. Distilled water may be substituted for deionized water at any step.
b. Background staining may increase if the Congo Red working solution is applied for more than 25 minutes.
c. Dehydrating alcohols must be eosin free, as eosin may result in nonspecific birefringence.

Expected Results

Conventional light microscopy
Amyloid, eosinophils, elastin — dull to brick red
Nuclei — purple/blue

Polarized microscopy
Amyloid — apple green birefringence

Apple green birefrigence detected by polarized microscopy is considered the most specific indicator of amyloid.

Massive, long standing amyloid deposits may display diminished birefringence as compared to newly formed deposits.

Recommended Controls
Tissue sections containing amyloid must be used.

A loss of staining intensity has been reported to occur in tissue sections stored for an extended period of time.

References

Date of Issue
June 2010
Special Stain Kit
Congo Red, Amyloid Stain

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Congo Red Solution (Item No. 38016SS9A, 500 mL)

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<td>Ethanol</td>
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<td>Isopropanol</td>
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<tr>
<td>Methanol</td>
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<td>Sodium Chloride</td>
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<td>Congo Red</td>
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Potassium Hydroxide Solution (Item No. 38016SS9B, 100 mL)

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Gill II Hematoxylin (Item No. 38016SS4C, 500 mL)

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<td>Ethylene Glycol</td>
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<td>Aluminum Sulfate</td>
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<td>Sodium Iodate</td>
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<td>Acetic Acid</td>
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