INTENDED USE

Amyloid Stain, Congo Red, kit is for use in the determination of amyloid in tissue sections. Both a standard procedure and a microwave procedure are provided. Amyloid Stain reagents are for "In Vitro Diagnostic Use".

Amyloid, an abnormal protein product of an immunologic disturbance, accumulates between parenchymatous cells of connective tissue resulting in amyloidosis. Amyloid may be detected in tissue with Congo red, a metachromatic anionic dye. Initial methods using Congo red required examination of stained sections with a polarizing microscope.  Puchtler’s modification, on which the Sigma-Aldrich procedure is based, intensifies Congo red staining by pretreatment of tissue with an alkaline solution of sodium chloride. The section is then evaluated with a light microscope or polarizing microscope.

An amyloid stain technique for rapid staining in microwave ovens is included. 1, 2

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

<table>
<thead>
<tr>
<th>Material</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloid Stain, Congo Red, kit</td>
<td>HT601-500 ml</td>
</tr>
<tr>
<td>Sodium Chloride Solution, Alcoholic</td>
<td>HT602-12 ml</td>
</tr>
<tr>
<td>Sodium Hydroxide Solution</td>
<td>HT603-500 ml</td>
</tr>
<tr>
<td>Congo Red Solution</td>
<td>HT604-500 ml</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Catalog No. A2693</td>
</tr>
<tr>
<td>Absolute Ethanol</td>
<td>Catalog No. A2694</td>
</tr>
<tr>
<td>Hematoxylin Solution</td>
<td>Catalog No. A2424</td>
</tr>
<tr>
<td>Mayer’s Hematoxylin Solution</td>
<td>Catalog No. TTR02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Absolute</td>
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<tr>
<td>Microwave Oven</td>
<td></td>
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<tr>
<td>Staining Jars, Catalog No. S5641</td>
<td></td>
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<tr>
<td>Staining Jars, Case, Catalog No. A2695</td>
<td></td>
</tr>
<tr>
<td>Microscope or polarizing microscope</td>
<td></td>
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</tbody>
</table>

REAGENTS

SOdium CHloride SolutIon, ALCoholic, Catalog No. HT601-500 ml
SD 3A alcohol, 80%, saturated with sodium chloride.

SOdium HYDroxide SolutIon, Catalog No. HT602-12 ml
Sodium hydroxide, 1% (0.25 mol/l).

Congo Red SolutIon, Catalog No. HT603-500 ml
Congo red (certified), 0.2%, in SD 3A alcohol, 80% saturated with sodium chloride.

STORAGE AND STABILITY:

Amyloid Stain reagents should be stored at room temperature (18–26°C). Congo Red Solution and Sodium Chloride Solution, Alcoholic, are stable until expiration date shown on labels.

Molarity of Sodium Hydroxide Solution can be verified by titration. Presence of precipitate in Sodium Hydroxide Solution, Alcoholic, or Congo Red Solution does not affect use.

PREPARATION:

Sodium Hydroxide Solution is supplied ready for use. Alcohol.
Alkaline Sodium Chloride Solution is prepared by adding 0.4 ml Sodium Hydroxide Solution to 40 ml Sodium Chloride Solution, Alcoholic. Mix just before use. Use once and discard.
Alkaline Congo Red Solution is prepared by adding 0.4 ml Sodium Hydroxide Solution to 40 ml Congo Red Solution. Filter solution and use within 15 minutes.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

Amyloid TISSUE-TROL™ control slides are paraffin embedded human tissue containing amyloid and should be considered potentially infectious.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Paraffin embedded tissue fixed in Carnoy’s fluid, absolute alcohol, Formalin Solution, 10% Neutral Buffered, or Bouin’s Solution, may be used. 2

Cut sections 6–12 microns. Thinner sections may not show small amounts of amyloid deposits and will not exhibit birefringence.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Positive control slides, such as Amyloid TISSUE-TROL™, Catalog No. A2424 or Catalog No. TTR02, should be included in each run

Mayer’s Hematoxylin Solution or Hematoxylin Solution, Gill No. 3
Xylene or xylene substitute
Ethanol, Absolute

FOR MICROWAVE PROCEDURE ONLY:

Microwave Oven
Staining Jars, Catalog No. S5641

OPTIONAL REAGENTS/EQUIPMENT:

Microscope or polarizing microscope

NOTES:

The use of polarized light is highly recommended for the evaluation of amyloid deposits. Apple-green birefringence is considered specific for amyloid.

Prolonged fixation in formalin may diminish Congo Red staining intensity. 1

Alcohol fixed tissue may yield more intense staining of amyloid, as compared to formalin fixed tissue. 2

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnosis tests or information.

PERFORMANCE CHARACTERISTICS

AMYLOID

Red to Pink-Red

NUCLEAR

Blue

ELASTIC FIBERS

Lighter Red

When examined under polarizing microscope, amyloid shows apple-green birefringence. If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES