
INTENDED USE

Sigma-Aldrich 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.¹ 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.² The section is then evaluated with a light microscope or polarizing microscope.

An amyloid stain technique for rapid staining in microwave ovens is included.^{3,5}

REAGENTS

SODIUM CHLORIDE SOLUTION, ALCOHOLIC, Catalog No. HT60-1
SD 3A alcohol, 80%, saturated with sodium chloride.

SODIUM HYDROXIDE SOLUTION, Catalog No. HT60-2
Sodium hydroxide, 1% (0.25 mol/l).

CONGO RED SOLUTION, Catalog No. HT60-3
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.

Alkaline Sodium Chloride Solution is prepared by adding 0.4 ml Sodium Hydroxide Solution, Catalog No. HT60-2, to 40 ml Sodium Chloride Solution, Alcoholic, Catalog No. HT60-1. Mix just before use. Use once and discard.

Alkaline Congo Red Solution is prepared by adding 0.4 ml Sodium Hydroxide Solution, Catalog No. HT60-2 to 40 ml Congo Red Solution, Catalog No. HT60-3. 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 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.

US Risks and Safety Statements

Alcoholic Sodium Chloride Solution is **FLAMMABLE** and **HARMFUL**. Harmful by inhalation, in contact with skin and if swallowed. Harmful: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Sodium Hydroxide Solution is **CORROSIVE**. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Congo Red Solution is **FLAMMABLE** and **TOXIC**. Highly flammable. May cause cancer. Harmful by inhalation, in contact with skin and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - no smoking. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing, gloves and eye/face protection. Restricted to professional users. Attention: Avoid exposure - obtain special instructions before use.

Reagent Alcohol is **FLAMMABLE** and an **IRRITANT**. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

Xylene is **FLAMMABLE** and **HARMFUL**. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Mayer's Hematoxylin Solution is **TOXIC**. Toxic if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing and gloves. Target organs: Nerves and liver.

Gill Hematoxylin Solutions are **HARMFUL**. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Liver and kidneys.

EU Risks and Safety Statements (Caution: Substances not yet fully tested)

Alcoholic Sodium Chloride Solution is **HIGHLY FLAMMABLE** and **HARMFUL**. Highly flammable. Harmful by inhalation, in contact with skin and if swallowed. Harmful: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed. Keep container tightly closed. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves.

Sodium Hydroxide Solution is an **IRRITANT**. Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Congo Red Solution is **HIGHLY FLAMMABLE** and **TOXIC**. Highly flammable. May cause cancer. Harmful by inhalation, in contact with skin and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - no smoking. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing and gloves. Restricted to professional users. Attention: Avoid exposure - obtain special instructions before use.

Reagent Alcohol is **HIGHLY FLAMMABLE** and an **IRRITANT**. Highly Flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Xylene is **HARMFUL**. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

Mayer's Hematoxylin Solution is **HARMFUL**. Harmful if swallowed.

Gill Hematoxylin Solutions are **HARMFUL**. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. 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 Sigma Amyloid TISSUE-TROL, Catalog No. A 2424, should be included in each run
Mayer's Hematoxylin Solution or
Hematoxylin Solution, Gills No. 3
Xylene
Ethanol, Absolute

FOR MICROWAVE PROCEDURE ONLY:

ACCUMATE™ H2100 Microwave Oven, Catalog Nos. A 9084 (110 v), or A 9209 (220 v)
Staining Jars, Catalog No. S 5641

OPTIONAL REAGENTS/EQUIPMENT:

Microscope or polarizing microscope

NOTES:

If the Sigma-Aldrich H2100 Microwave Oven is used, please see the Owner's Manual for instructions.

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.⁷

Alcohol fixed tissue may yield more intense staining of amyloid, as compared to formalin fixed tissue.⁸

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

PROCEDURE:

1. Deparaffinize and hydrate to deionized water.
2. Stain in Mayer's Hematoxylin Solution, for **10 minutes**.
OR
Hematoxylin Solution, Gill No. 3, for **3 minutes**.
3. Rinse in tap water for **5 minutes**.
4. Place in Alkaline Sodium Chloride Solution for **20 minutes**.
5. Stain in Alkaline Congo Red Solution for **20 minutes**.⁶
6. Rinse in 3 changes of absolute ethanol.
7. Clear in xylene and mount. Examine microscopically.

ACCUMATE™ H2100 MICROWAVE PROCEDURE:

1. Deparaffinize and hydrate to deionized water.
2. Place slides in **40 ml** of Hematoxylin Solution, Gill No. 3, contained in a plastic Coplin jar. Loosely cover jar with lid, or use lids with holes drilled into them.
3. Microwave at **800 watts** for **5 seconds**.
4. Rinse well in running water for **1–2 minutes**.
5. Place slides in **40 ml** Alkaline Sodium Chloride Solution contained in a plastic Coplin jar.
6. Microwave at **400 watts** for **20 seconds**.
7. Place slides directly into **40 ml** of Alkaline Congo Red Solution contained in a plastic Coplin jar.
8. Microwave at **400 watts** for **30 seconds**. Let incubate for **5 minutes**.
9. Rinse in three changes of absolute ethanol.
10. Clear in xylene and mount. Examine microscopically.

PERFORMANCE CHARACTERISTICS

- Amyloid - Red to Pink-Red
- Nuclei - 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

1. Selected Histochemical and Histopathological Methods. Edited by SW Thompson, Charles C. Thomas, Springfield (IL), 1966, pp 402-405
2. Theory and Practice of Histotechnology. Edited by DC Sheehan and BB Hrapchak, 2nd ed. Mosby, St. Louis (MO), 1980, pp 177-178
3. Leong AS-Y, Milios J: Rapid immunoperoxidase staining of lymphocyte antigens using microwave irradiation. J Pathol 148:183, 1986
4. Brinn NT: Rapid metallic histologic staining using the microwave oven. J Histotechnol 6:125, 1983
5. Valle S: Special stains in microwave oven. J Histotechnol 9:237, 1986
6. Theory and Practice of Histological Techniques. Edited by J D Bancroft and A Stevens, Churchill Livingstone, New York (NY), 1977, p 129
7. Theory and Practice of Histological Techniques, 5th Edition, Bancroft JD, Gamble M, Editors Churchill Livingstone, New York (NY), 2002, p 311
8. Humanson's Animal Tissue Techniques, 5th Edition, Presnell JK and Schreiberman MP, Editors The John Hopkins University Press, Baltimore (MD), 1997, p 270

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