

Congo Red Stain

PRODUCT INFORMATION:
 SSP002 100ml Ready to use
 SSP002 250ml Ready to use
 SSP002 500ml Ready to use

PERFORMANCE CHARACTERISTICS:
Staining Interpretation:
Nuclei: Dark Blue
Amyloid:
 In Transmitted light – Pink to Red
 In Polarized light – Apple green birefringence
Connective tissue; Collagen: Light Red

SUMMARY AND EXPLANATION

For laboratory use only

The Congo Red Stain[™] is intended for use as a qualitative histological stain to selectively demonstrate Amyloid in formalin-fixed, paraffin-embedded tissue. It is a ready-to-use staining kit and this product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information and proper controls.

PRINCIPLE OF THE PROCEDURE

Amyloid is a homogenous structure made up of protein fibrils (each between 8 and 15nm in diameter) that can be stained eosinophilically, e.g., in the case of amyloidosis, Amyloid deposits are formed in the intercellular spaces. All deposits of amyloid contain similar protein fibrils that are resistant to the body's natural defense mechanisms and once they have formed cannot be eliminated.

Congo Red is a metachromatic anionic dye and it forms the hydrogen bridge bonds with carbohydrate component of the substrate. The beta pleated sheets of amyloid are suitable in size and shape to accommodate the congo red molecules, which are held in the lattice work of the beta pleated sheets. The tissue stained with congo red appears pink-red under the transmitted-light microscope and a brilliant apple green birefringence under the polarized light due to conspicuous dichroism. Congo red may also stain unexpected structures such as; keratin, elastic and dense collagen fibers which cannot be visualized under polarized light.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack sizes		
			100ml	250ml	500ml
Congo Red Stock Solution (Reagent A)	IPS019	RT	100ml	250ml	500ml
1% NaOH (Reagent B)	IPS024	RT	2ml	5ml	10ml
Alkaline Alcohol Solution (Reagent C)	IPS020	RT	100ml	250ml	500ml
Modified Mayer's Hematoxylin (Reagent D)	PS020	RT	100ml	250ml	500ml

STORAGE AND HANDLING

Storage Recommendations: Store at Room Temperature. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after expiration date provided on the vial.**

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the bottles at room temperature away from sunlight in an upright position.

PREPARATION OF CONGO RED WORKING SOLUTION

For 10ml of Congo Red Working Solution: Measure 9.9ml of Reagent A (Congo Red Stock Solution) and add 0.1ml of Reagent B (1% NaOH).

Note: Filter the solution and use it before 20min as the solution may degrade in long stand.

DS-SSP002-B

SPECIMEN PREPARATION

Recommended positive controls:

Formalin-fixed, paraffin-embedded or frozen tissue sections containing amyloid deposits.

Sample preparation and fixation:

- Fixation plays an important role in preserving the tissue structure to be visualized using the stain.
- Carnoy's fluid, Absolute alcohol, 10% Neutral buffered formalin, Bouin's solution may be used for fixation.
- Ensure that the fixed sections are adequately embedded in paraffin.
- Cut the sections, usually 6-12 µm to show smaller amyloid deposits which can exhibit birefringence under polarized light.

PRECAUTIONS

1. Normal precautions exercised in handling laboratory reagents should be followed
2. This product should be used by qualified and trained professional users only
3. The product contains alcohol and is classified as highly-flammable, must be kept away from ignition sources
4. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information
5. Dispose of waste observing all local, state, provincial or national regulations
6. Do not use reagents after expiration date
7. Use protective clothing and gloves, while handling reagents
8. Avoid microbial contamination of reagents as it may lead to incorrect results

MATERIALS REQUIRED, BUT NOT PROVIDED

- Xylenes
- Graded Alcohols (50%, 70%, 95%, Absolute)
- Bluing Solution (Optional)
- Tap Water
- Distilled Water
- DPX Mountant
- Microscopic Slides (Positively charged)
- Slide Holder
- Polarized Lens
- Cover Slips
- Coplin Jars
- Drying Oven

STAINING PROCEDURE

1. Bake the human FFPE tissue sections at 70°C in hot air oven for 20 minutes.
2. Deparaffinize and hydrate the sections using xylenes, graded alcohols - 80%, 70%, 50% and distilled water for 2 minutes each.
3. Stain the sections using Congo Red working solution for 10 minutes.
4. Rinse in distilled water for 1-2 minutes.
5. Differentiate quickly in Alkaline Alcohol solution (Reagent C) 9-10 dips.
6. Rinse in tap water for 2 minutes.
7. Counter stain with Modified Mayer's Hematoxylin (Reagent D) for 30-60 seconds.
8. Rinse the slides under running tap water for 2 minutes.
9. Place the slides in distilled water for 10 minutes.
10. Dehydrate rapidly using graded alcohols.
11. Clear the sections using 2 changes of xylene.
12. Cover slip the sections using DPX mountant.

QUALITY CONTROL

The recommended positive tissue control for Congo Red stain is tissue sections containing amyloid deposits.

PERFORMANCE CHARACTERISTICS

Congo Red for Nuclei stains blue color, Amyloid - In transmitted light appears Pink to Red in color and In polarized light appears Apple green birefringence in color and Connective tissue and Collagen stains Light Red in color.

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, reagent trapping or inaccurate results
3. Do not allow the section to dry out during the entire staining process
4. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

Authorized and skilled personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

BIBLIOGRAPHY

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3. Selected Histochemical and Histopathological Methods. Edited by SW Thompson Charles C, Thomas, Springfield (IL), 1966, pp 402-405.
4. Theory and practice of Histotechnology. Edited by DC Sheehan and BB Hrapchak, 2nd ed. Mosby, St. Louis (MO), 1980, pp177-178.
5. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for use in Biology and Medicine, 10th Edition. (ed. Horobin, R.W. and Kiernan J.A), Bios, 2002.

EXPLANATION OF SYMBOLS

 Lot number / Batch number



Expiry

RT- Room Temperature

Laboratory Use Only