PathnSitu Biotechnologies

Congo Red (NA) Stain Kit

Laboratory Use Only

PRODUCT INFORMATION:

REF

SSP019-NA 100ml
SSP019-NA 250ml
SSP019-NA 500ml

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 (NA) Stain Kit" is designed as a qualitative histological stain to selectively demonstrate amyloid in formalin-fixed, paraffin-embedded tissue. This product is not intended for diagnostic or therapeutic use. Results should be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

Amyloid is a homogeneous structure composed of protein fibrils, each measuring between 8 and 15 nm in diameter, which can be stained eosinophilically. For example, in amyloidosis, amyloid deposits form in intercellular spaces. All amyloid deposits contain similar protein fibrils that resist the body's natural defences and, once formed, cannot be eliminated.

PRINCIPLE OF THE PROCEDURE

Congo Red is a metachromatic anionic dye that forms hydrogen bonds with the carbohydrate component of the substrate. The beta pleated sheets of amyloid are suitably sized and shaped to accommodate Congo Red molecules, which are held within the lattice of the beta pleated sheets. Tissue stained with Congo Red appears pink-red under the transmitted-light microscope and displays a brilliant apple-green birefringence under polarized light due to its conspicuous dichroism. Congo Red may also stain unexpected structures such as keratin, elastic fibers, and dense collagen fibers, which cannot be visualised under polarized light.

REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack sizes		
			100ml	250ml	500ml
Congo Red Stock (Reagent A)	IPS019A	RT	1ml	2.5ml	5ml
NaOH-A (Reagent B)	IPS024	RT	2ml	5ml	10ml
Alkaline Alcohol Solution (Reagent C)	Not Provided- Refer to Reagent Preparation				
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 the expiration date provided on the vial.**

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

During transport, short-term exposure to temperatures between 2-8 °C does not affect product performance.

SPECIMEN PREPARATION

Sample preparation and fixation:

- Fixation plays an essential role in preserving the tissue structure to be visualized using the stain.
- 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 6-12microns 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. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
4. Dispose of waste observing all local, state, provincial or national regulations.
5. Do not use reagents after the expiration date
6. Use protective clothing and gloves while handling reagents
7. Avoid 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)
- DPX Mountant
- Microscopic slides (positively charged)
- Slide holder
- Polarized lens
- Cover slips
- Coplin jars
- Alkaline Alcohol Solution

PREPARATION OF WORKING SOLUTION

Preparation of Congo Red Stock Solution 1:

Components	Quantity Required		
Pack Size	100ml	250ml	500ml
Congo Red Stock (Reagent A)	1ml	2.5ml	5ml
80% Alcohol (Not provided)	99ml	247.5ml	495ml

Preparation of Congo Red Working Solution:

For 10ml of Congo Red Working Solution: Measure 9.9ml of Congo Red Stock Solution 1 (Refer to reagent preparation above) and add 0.1ml of Reagent B, NaOH-A).

Note: Filter the solution and use it within 20 minutes, as it may degrade over time.

Preparation of Alkaline Alcohol Solution (Reagent C):

Components	Quantity Required		
	100ml	250ml	500ml
50% Alcohol (Not provided)	99ml	247.5ml	495ml
NaOH-A (Reagent- B)	1ml	2.5ml	5ml

Note: Once the stock reagents are prepared, they remain stable until the expiration date of the kit.

US Office: 7026 Koll Center Parkway, STE#211, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
India Office:PS Heights, Plot# A-28/1/11B/B, Road#, 15, Industrial Development Area, Nacharam, Secunderabad-76
Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com

STAINING PROCEDURE:

1. Bake the human FFPE tissue sections at 70°C 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 with Congo Red Working solution (refer to the working solution preparation above) for 10 minutes.
4. Rinse in distilled water for 1-2 minutes.
5. Differentiate quickly in Alkaline Alcohol Solution (Reagent C) – (refer to reagent preparation above) for 9-10 seconds.
6. Rinse in tap water for 2 minutes.
7. Counterstain 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 with two changes of Xylene.
12. Coverslip 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 (NA) Stain Kit stains the **Cell Nuclei** in **blue** colour, **Amyloid** - In transmitted light appears **Pink to Red** in colour and in polarized light appears **Apple green** birefringence and **Connective tissue** and **Collagen** stains **Light Red** in colour

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to the 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. Gently mix all the reagents prior to use.
5. Excessive or incomplete counterstaining may compromise the interpretation of the results.
6. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorised, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of the results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. In instances where the staining pattern or localisation differs from the specifications outlined in this datasheet, please get in touch with technical support for guidance.
11. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. NaOH is strongly caustic and unstable in air (absorbs CO_2); incorrect concentration alters Congo Red binding and reduces staining quality.
14. This product is intended for single-use application only. Once applied to a tissue section, reagents should not be recovered or reused, as this may compromise test integrity and specificity.
15. PathnSitu makes no warranties beyond those expressly stated in the product description.
16. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
17. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
18. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

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	Lot number / Batch number		Expiry
°C	Storage limitation	RT	Room Temperature
	Date of manufacture	REF	Catalogue number
	Manufacturer address		

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