Congo Red Staining Kit

REF 860-026

05279429001

IVD Σ 40

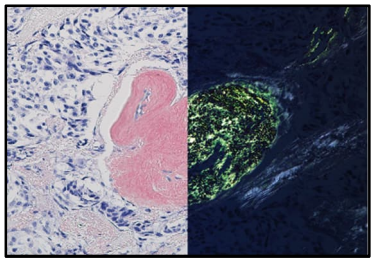

Figure 1. Congo Red Staining Kit staining amyloid in lung tissue, bright field (left) and polarized light (right).

INTENDED USE

Congo Red Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate amyloid in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark Special Stains instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

Congo Red Staining Kit is a modification of the Highman's technique.[1]

In 1922, the use of Congo red dye for detecting amyloid was discovered by Bennhold.[2] The dye hydrogen bonds to β-pleated sheets of amyloid and is known to bind to all forms of amyloid.[2] Mature amyloid contains fibrils that range in size from 0.1 to 10µm long that assemble to form insoluble plaques that are resistant to degradation.[3]

The accumulation of insoluble amyloid fibrils in extracellular spaces can lead to amyloidosis.[4,5] There are several types of amyloidosis and each is characterized by the presence of insoluble amyloid plaque(s) and/or masses, which can lead to cell damage and disrupt normal organ function.[2,4]

Congo Red Staining Kit demonstrates amyloid to aid the pathologist in the diagnosis of amyloidosis.

PRINCIPLE OF THE PROCEDURE

The staining reaction is based on the application of Congo Red Stain, which stains the patterns of atypical proteins (amyloid) pink to salmon. Congo Red Buffer is added to differentiate the stain. Congo Red Hematoxylin, a Mayer's hematoxylin solution, is used to provide contrasting blue to purple nuclear staining. The β-pleated sheets of amyloid are suitable in size and shape to accommodate the Congo red molecules, which are held in the latticework of the β-pleated sheets.[6] Birefringence is an intrinsic property of the amyloid fibril Congo red complex.[7,8]

This kit is optimized for use on the BenchMark Special Stains instruments. The reagents are applied to tissue on microscope slides and mixed over the entire specimen.

MATERIAL PROVIDED

The reagent vials are supplied in barcode labeled carriers to insert into the reagent tray of the instrument. Each kit contains sufficient reagents for 40 tests.

One 19 mL vial of Congo Red Stain contains approximately 0.1% Congo red and 70% isopropanol.

One 13 mL vial of Congo Red Buffer contains approximately 0.5% glycine and 2.0% sodium chloride.

One 19 mL vial of Congo Red Hematoxylin contains modified Mayer's hematoxylin.

Three vial inserts with sipping straws.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution or titration is required. Further dilution of any of the reagents may result in unsatisfactory staining.

The reagents in this kit have been optimally diluted for use on the BenchMark Special Stains instruments.

MATERIALS REQUIRED BUT NOT PROVIDED

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. BenchMark Special Stains instrument
4. BenchMark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
5. BenchMark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
6. BenchMark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
7. General purpose laboratory equipment

STORAGE AND STABILITY

Congo Red Staining Kit should be stored at 2-8°C. Refrigerated kit components should be brought to room temperature prior to use.

When properly stored, unopened and opened reagents are stable to the date indicated on the label. Do not use reagent beyond the expiration date indicated on the kit.

There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows a decrease in staining as it could indicate reagent instability.

SPECIMEN PREPARATION

Routinely processed, FFPE tissues are required for use with this product and BenchMark Special Stains instruments. The recommended tissue fixative is 10% neutral buffered formalin.[1]

Perform specimen collection and storage according to CLSI document M29 T2.[9] Cut sections to the appropriate thickness, approximately 8 µm, and place the sections on positively charged glass slides.

1. Dry the slides.[1]
2. Print appropriate barcode label(s).
3. Apply barcode labels to the frosted end of the slides prior to loading the slides onto the instrument (see instrument User Guide for correct application of labels).

Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains instrument.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[10,11]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, rinse with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark Special Stains instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Danger 	H225	Highly flammable liquid and vapour.
	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	H336	May cause drowsiness or dizziness.
	H350	May cause cancer.
	H373	May cause damage to organs through prolonged or repeated exposure.
	P201	Obtain special instructions before use.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P260	Do not breathe mist or vapours.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P308+ P313	IF exposed or concerned: Get medical advice/ attention.
	P370+ P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.

EUH208: This product contains Sodium iodate. May produce an allergic reaction.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

Before first use, a vial insert and sipping straw must be placed in the reagent vial.

Remove the shipping cap from the vial and place the insert and straw into the vial. The insert and sipping straw should be left in the vial, once the vial has been opened.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Perform the staining run according to the recommended protocol in Table 2 and the instructions in the User Guide.
4. When the run is complete, remove the slides from the instrument.
5. Use the soft cap to cover the reagent vial when reagent is not in use.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

The following procedures allow flexibility to accommodate user preference. This product has been optimized for use with BenchMark Special Stains instruments but the user must validate results obtained with this product.

Table 2. Recommended staining protocol for Congo Red Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S Congo Red
Protocol Step	Method
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 4 minutes is recommended.
Option 1 or Option 2	The default is Option 1. Select Option 1 or Option 2: • Option 1 will dispense Congo Red Stain before Hematoxylin, nuclei will be purple. • Option 2 will dispense Hematoxylin before Congo Red Stain, nuclei will be blue.
Option 1	
Optimize Stain Intensity (C. Red Stain)	The Option 1 default is 37°C for 24 minutes. Select to enable adjustment of staining intensity.* Select temperature from 37 - 60°C: • 37°C, lighter staining of amyloid 60°C, darker staining of amyloid Select an incubation time from 20 - 32 minutes: • 20 minutes, lighter staining of amyloid • 32 minutes, darker staining of amyloid
Optimize Hematoxylin Intensity (C. Red Hematoxylin)	The Option 1 default is 12 minutes. Select to optimize hematoxylin*: • 4 minutes, lighter nuclear staining • 16 minutes, darker nuclear staining
Option 2	
Optimize Hematoxylin Intensity (C. Red Hematoxylin)	The Option 2 default is 8 minutes. Select to optimize hematoxylin*: • 4 minutes, lighter nuclear staining 16 minutes, darker nuclear staining
Optimize Congo Red Intensity (C. Red Stain)	The Option 2 default is 48 minutes. Select to enable adjustment of staining intensity.* Select an incubation time from 32 - 48 minutes: • 32 minutes, lighter staining of amyloid 48 minutes, darker staining of amyloid

* To adjust staining preferences, increment the stain temperature and incubation time one parameter at a time.

Recommended Post-Instrument Processing

1. Rinse slides in three changes of 100% ethanol to remove leftover solution, for 5 to 10 seconds each with gentle agitation.
2. Dehydrate slides in three changes of 100% xylene, for 5 to 10 seconds each with gentle agitation.
3. Coverslip with permanent mounting media.

4. Compatible with the VENTANA HE 600 system coverslipping protocol. For further instructions, refer to the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURE

An example of a positive control material would be FFPE human tissue positive for amyloid, such as that from a patient with amyloidosis.[1] Control tissue should be fresh autopsy, biopsy, or surgical specimen prepared or fixed as soon as possible in a manner identical to test sections. Such tissues should monitor all steps of the analysis, from tissue preparation through staining.

Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue processing. The cellular components of other tissue elements may serve as the negative control.

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

The control tissue must be tested with each run.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples.

If the positive tissue components fail to demonstrate positive staining, results with the test specimens should be considered invalid. If the negative components demonstrate positive staining, results with patient specimens should also be considered invalid.

Unexplained discrepancies in control results should be referred to the local support representative immediately. If quality control results do not meet specifications, patient results are invalid. The cause must be identified and corrected, and the patient samples repeated.

STAINING INTERPRETATION / EXPECTED RESULTS

The Congo Red Staining Kit was tested to demonstrate amyloid.

- Amyloid (under bright field): pink to salmon
- Amyloid (under polarized light): apple-green birefringence
- Nuclei: purple with protocol Option 1
- Nuclei: blue with protocol Option 2
- Amyloid may appear as circular or ribbon-like pink staining deposits throughout the tissue. It has a particular predilection for deposition in blood vessel walls and basement membranes. Keratin, elastic and dense collagen fibers may retain the Congo red dye. Birefringence is an intrinsic property of the amyloid fibril Congo red complex.[7,8] Without both the pink to salmon staining of Congo red under bright field and the apple-green birefringence under polarized light a definite identification of amyloid cannot be made.

SPECIFIC LIMITATIONS

Only positively charged microscope slides have been used and validated for this assay.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity was evaluated in amyloid positive tissues (various tissue types) and normal skin. Both protocol options (Option 1 and Option 2) were tested on all tissue cases listed in Table 3. All evaluated tissue cases (60/60) passed for acceptable staining as shown in Table 3.

Table 3. Sensitivity/Specificity of Congo Red Staining Kit was determined by testing the following FFPE normal and diseased tissues.

Tissue	# passed / # tested
Amyloid-positive tissues (various tissue types*)	45 / 45
Normal skin	15 / 15

*Tissues tested include but are not limited to thyroid, prostate gland, liver, stomach, colon, soft tissue and lung.

Precision

Precision of Congo Red Staining Kit was determined across multiple runs, days, instruments, and reagent lots using multiple cut slides from various amyloid positive tissues (thyroid, prostate gland, liver, stomach, colon, soft tissue and lung) using protocol Option 1. All acceptance criteria were fully met. Precision studies were performed for the Congo Red Staining Kit according to Table 4.

Table 4. Precision slide studies for Congo Red Staining Kit.

Parameters Tested	# of conditions	# passed / # tested
Run to Run	3 runs, same day	54 / 54
Day to Day	5 days	90 / 90
Instrument to Instrument	3 instruments	54 / 54
Intra Run	same day, same instrument	54 / 54
Lot to lot	3 lots	54 / 54

The results demonstrated no significant difference in staining intensity among the slides.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. If tissue sections wash off the slide, confirm the slides are positively charged.
7. Thicker sections demonstrate amyloid deposits more efficiently, while thinner sections may not show the apple-green birefringence.
8. Staining intensity decreases with the age of the sections; freshly cut sections give a more intense staining reaction.
9. It is important to dehydrate slides thoroughly and evaluate promptly. As with manual staining, the Congo red may be extracted during prolonged exposure to alcohol during dehydration.
10. Extended stay of the slides on-board the instrument after run completion may affect quality and intensity of the staining. If the staining is inappropriate, remove slides promptly at the end of the run and proceed to post-instrument processing.
11. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

1. Carson FL, Cappellano C. HIstotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
2. Gilbertson JA. Amyloid. In: Bancroft's Theory and Practice of Histological Techniques.2019:231-253.
3. Dapson RW. Amyloid from a Histochemical Perspective. A Review of the Structure, Properties and Types of Amyloid, and a Proposed Staining Mechanism for Congo Red Staining. Biotech Histochem. 2018;93(8):543-556.
4. Mollee P, Renaut P, Gottlieb D, et al. How to Diagnose Amyloidosis. Intern Med J. 2014;44(1):7-17.
5. Gertz MA, Dispenzieri A. Systemic Amyloidosis Recognition, Prognosis, and Therapy: A Systematic Review. JAMA. 2020;324(1):79-89.
6. Wheater PR, Burkitt HG. Basic Histopathology, 2nd edition. New York: Churchill Livingstone; 1991.
7. Navarro A, Tolivia J, Valle E. Congo Red Method for Demonstrating Amyloid in Paraffin Sections. J Histotechnol. 1999;22(4):305-308.

8. Fredenburgh JL, Grizzle WE. Special Stains: Their Chemical Mechanism. Chicago: ASCP Press; 1998.
9. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
10. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
11. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
H	Updates to the Warnings and Precautions and References sections.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

CE