BIOGNOST®

CONGO RED REAGENT

IVD *In vitro* diagnostic medical device CE

For use with Congo Red Highman kit

INSTRUCTIONS FOR USE

REF Catalogue number: CR-OT-100 (100 mL)

Introduction

Congo Red reagent is a main component of Congo Red Highman kit. This kit is used for staining amyloids (amorphous clusters). Amyloid deposits are stained characteristically red, but under polarized light they display double refraction and provide green coloration.

Product description

- **CONGO RED REAGENT -** Alcoholic solution with optimal concentration of Congo Red dye.

Example of using Congo red reagent as a component of Congo Red Highman kit

Other slides and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro.
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's reagents that comprise Congo Red Highman kit: Potassium hydroxide, solution (cat. number: KHO-OT-100), Hematoxylin G2 (cat.number: HEMG2-OT-100)

Preparing the histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Section the paraffin block to **8-10** μm sections and mount them on VitroGnost glass slide (mount the sections to VitroGnost adhesive glass slide)

NOTE

Immerse the section in reagent completely.

Sample staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Immerse in Congo Red reagent	5 min
6.	Drain the reagent off the section without rinsing	
7.	Immerse in Potassium hydroxide, solution	15 seconds
8.	Rinse under tap water	5 dips
9.	Immerse in Hematoxylin G2 reagent	3 min
10.	Rinse under tap water	3 min
11.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
12.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
13.	Dehydrate using 100% alcohol (Histanol 100)	2 min
14.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost cover glass.

Result

Amyloid deposits - pink to red; green under polarized light
Nuclei - blue

Note

By using polarized light microscope amyloid clusters demonstrate double refraction of light (green).
Staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date

Keep Congo Red reagent in a tightly closed original package at a temperature of +15 to +25 °C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.
2. Highman, B., Improved methodes for demonstrating amyloid in paraffin sections, Archives of Pathology, V 41, p. 559

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package	REF	Product code		European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile						