

CONGO RED PUCHTLER KIT

IVD In vitro diagnostic medical device



Three-reagent amyloid deposits staining kit

INSTRUCTIONS FOR USE

REF Catalog number: CRP-100T (for staining up to 100 sections)

CRP-K-100 (3x100 mL + 1x30 mL)

Introduction

Congo Red Puchtler kit is used for staining amyloids (amorphous clusters) in histology sections. Despite their variable protein composition, amyloid deposits are linked by fibril beta-sheets formation. Congo Red dye binds to fibrils and form a regular pattern of dye molecules during the process. The structural regularity of the dye under polarized light shows double refraction of light by displaying green coloration. High pH level and ionic strength of solutions according to Puchtler achieve higher dye specificity to amyloid.

Product description

- **CONGO RED PUCHTLER KIT** - Three-reagent amyloid deposits staining kit

The kit contains:	For 100 tests (CRP-100T)	3x100 mL + 1x30 mL (CRP-K-100)
Congo Red, Puchtler reagent	CRPR-OT-100 (100 mL)	CRPR-OT-100 (2 x 100 mL)
Hematoxylin G1	HEMG1-OT-30 (30 mL)	HEMG1-OT-100 (100 mL)
Sodium hydroxide, solution	NH-OT-30 (30 mL)	NH-OT-30 (30 mL)

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 New, BioMount C, and Canada Balsam
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm

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 **5-10 μm** sections and mount them on VitroGnost glass slide (mount the sections thicker than 6 μm to VitroGnost adhesive glass slide)

Preparing Congo Red Puchtler working solution

Note: 100% ethyl alcohol is required for preparing working solution (BioGnost's Histanol 100)

In a glass staining jar (Coplin) mix **8 ml of Congo Red, Puchtler reagent** with **32 ml of 100% ethyl alcohol** (BioGnost's Histanol 100). Add **12 drops of Sodium hydroxyde, solution**.

For preparation of 100 ml of Congo Red Puchtler working solution mix **20 ml of Congo Red, Puchtler reagent** with **80 ml of 100% ethyl alcohol** (BioGnost's Histanol 100). Add **1 ml of Sodium hydroxyde, solution**.

Congo Red Puchtler working solution must be freshly prepared before use and discarded after use. Filter the reagents shortly before if necessary (pore size - 25 μm).

Note

- Staining procedure using Congo Red Puchtler working solution is recommended to be conducted in glass staining jar (Coplin jar) for **8 sections**.
- Staining procedure is conducted in digester.

Histological sections staining procedure:

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 10 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.	Add Hematoxylin G1 to the sections (≥ 5 drops)	5 min
6.	Rinse under tap water	4 min
7.	Immerse the sections in freshly prepared Congo Red working solution	18 min
8.	Rinse under tap water	until excessive dye is rinsed (a few minutes)
9.	Dehydrate using 70% alcohol (Histanol 70)	10 dips
10.	Dehydrate using 95% alcohol (Histanol 95)	10 dips
11.	Dehydrate using 100% alcohol (Histanol 100)	3 min
12.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 5 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 VitroGnost cover glass.

Result

Amyloid deposits - brick red, green under polarized light

Collagen - bright red, grey-silver under polarized light

Nuclei - blue

Note

By using polarized light amyloid clusters demonstrate double refraction of light (green). Correct amyloids display and recording using this technique depends on section thickness, adequate use of polarization, as well as digital format correction.

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. 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 Puchtler kit in a tightly closed original package at temperature between 15°C and 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. Elgetany, M.T., Saleem, A., Barr, K. (1989), The congo red stain revisited, Annals of Clinical and Laboratory Science, 19(3):190-5.

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				



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