CARMINIC ACID C.I. 75470

IVD *In vitro* diagnostic medical device CE

Natural Red 4

For staining mucicarmine and glycogen

INSTRUCTIONS FOR USE

REF Catalogue number: CARA-P-10 (5 g) CARA-P-10 (10 g) CARA-P-25 (25 g)

Introduction

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Carminic acid is a natural dye derived from Carmine color. It consists of anthraquinone that is bound to glucose sugar. Carminic acid is used for various staining procedures and visualization of glycogen and mucous substances, as well as for nuclear staining and vital staining.

Product description

- **CARMINIC ACID powder dye** - Powder dye for making solution for histology staining

Example of use of Carminic acid powder dye for detecting glycogen

Other sections and reagents that are used in the staining method:

- absolute ethyl alcohol, methanol
- potassium chloride, potassium carbonate
- ammonia
- Hematoxylin ML

Preparing the solutions for staining

1. Carminic acid stock solution
   - Dissolve 2 g of Carminic acid powder dye and 5 g of potassium chloride in 60 mL of distilled water while heating the mixture.
   - Add 1 g of potassium carbonate and let it slowly boil (the mixture produces excessive amount of foam). Let it boil for a few minutes as the color changes to dark red.
   - Let it cool and add 20 g of ammonia.
   - Close tightly and store in cool place. The solution is stable for 2 months.
2. Carminic acid working solution
   - Filter 20 mL of stock solution, add 30 mL of ammonia and 30 mL of methanol.
3. Differentiating solution
   - Mix 40 mL of methanol with 80 mL of ethyl alcohol and add 100 mL of distilled (demi) water.

Preparing 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 Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

Histological sections 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.	Stain using Hematoxylin ML.	3-5 minutes
6.	Rinse the section with tap water	3-5 minutes
7.	Stain in Carminic acid working solution	5-20 minutes
8.	Differentiate in differentiation solution, stop the procedure after the dye ceases to be released from the section	
9.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
10.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 1 minute each
11.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 minute each
12.	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

Glycogen - red
Mucosa, fibrin, amyloid - pink to red

Note

The mentioned formulation is only one of the ways of preparing the dye solution. Depending on personal requests and standard laboratory operating procedures, the dye solution can be prepared 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 Carminic acid powder dye in a tightly closed original package at temperature between 15°C and 25°C. Keep in dry places, do not freeze and avoid exposure to direct sunlight. Expiry date is stated on the product's label.

References

1. Conn, J. (1977): *Biological Stains*, 9th ed., Baltimore: Williams and Wilkens Co.

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