BIOGNOST®

AZOCARMINE G powder dye, C.I. 50085

IVD *In vitro* diagnostic medical device CE

Rosinduline

For staining according to AZAN trichrome method

INSTRUCTIONS FOR USE

REF Catalogue number: AZC-P-5 (5 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. Azocarmine G is anionic dye, very similar to Azocarmine B. It is primarily used with AZAN staining method (according to Heidenhain or various modifications). It is also used in histology polychromatic staining methods, and it is used in histochemistry to dim the background fluorescence in fluorescent staining using Schiff's reagent.

Product description

- **AZOCARMINE G -** Powder dye for making dye solution for use in histology and histochemistry

Example of use of Azocarmine G powder dye (according to Heidenhain AZAN staining method)

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

- concentrated acetic acid
- alcoholic aniline solution, acid alcohol, phosphomolybdic acid, Azan reagent (reagents from BioGnost's AZAN kit, product code AZT-100T, AZT-K-250)

Preparing the Azocarmine staining solution

- Add 2 g of Azocarmine G powder dye to 200 ml of distilled (demi) water, heat until it boils.
- Let it cool to room temperature.
- Filter the solution. Add 2 ml of concentrated acetic acid.
- Do not filter again.

The solution is stable for 1 year at room temperature (stir and heat to 56°C before use), that is, for two weeks if it constantly being kept at 56°C.

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 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

Pour the reagents (100 ml volumes) into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Heated Azocarmine solution must be first cooled down to room temperature and returned to the prepared original packaging. Close tightly.

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.	Heat Azocarmine solution to +56°C and immerse the sections. Cover the staining jar to avoid evaporation	30 min at +56 °C
6.	Cool the sections down at room temperature	5 min
7.	Rinse the section with tap water	until the excessive dye is washed off of the section (a few seconds)
8.	Add Aniline, alcoholic solution (≥ 5 drops) and differentiate	1 min
	Note: it is recommended to differentiate using microscopic examination of the section. Rinse the section with distilled (demi) water and examine the section microscopically. Repeat the step if necessary.	
9.	Add Acid alcohol (≥5 drops)	1 min
10.	Remove the reagent off the section without rinsing	
11.	Treat using Phosphotungstic acid, 5% solution: pour the solution into the staining jar and immerse the sections. Cover the staining jar to avoid evaporation	Treat intestinal sections and sections of small intestine (and similar tissues) for 30 minutes; treat the kidney sections for 60 minutes
12.	Remove the reagent off the section without rinsing	
13.	Stain using Azan reagent: pour the reagent in the staining jar and immerse the sections Cover the staining jar to avoid evaporation	Stain intestinal sections and sections of small intestine (and similar tissues) for 30 minutes; treat the kidney sections for 60 minutes
14.	Rinse in distilled (demi) water	2 exchanges, 5 dips each

15.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
16.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
17.	Dehydrate using 100% alcohol (Histanol 100)	2 min
18.	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

Nuclei, erythrocytes, acidophilic granules of the pituitary gland - red
Neurofibrils (neuroglia) - hues of red
Muscle fibers - pink to red-pink
Collagen, reticulin, basophilic cell membranes, renal glomerular stroma, basement membranes - blue to dark blue
Elastic fibers - no color

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 Azocarmine G 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 Wilkins Co.
2. Kiernan, J.A. (2008): Histological and Histochemical Methods. Theory and Practice. 4th edition, Bloxham, UK: Scion

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	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	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						