BIOGNOST®

ERIOCHROME CYANINE R, powder dye, C.I. 43820

IVD *In vitro* diagnostic medical device CE

Mordant Blue 3

For staining of nuclei

INSTRUCTIONS FOR USE

REF Catalogue number: ECR-P-10 (10 g)

Introduction

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve good visualization of tissue and cellular structures, the samples need to be properly stained. Eriochrome Cyanine R (also known as Solochrome Cyanine R) belongs to the group of anionic sulphonaphthalene mordant dyes. It can be used alone as a pH indicator or as a red anionic dye, however, as intense colored complexes are built with transition metal ions (such as iron ions), this dye is most often used as a synthetic replacement for hematoxylin. Namely, Eriochrome and ferric ions make up to four different complexes, two of which are red and two blue. If ferrous solutes of this dye are used under appropriate conditions, a selective staining of the nuclei can be obtained similar to the results of progressive staining with hematoxylin. This method is compatible with conventional eosin counterstaining, but also with many other histochemical methods. Eriochrome therefore represents a cost-effective and environmentally acceptable synthetic substitute for hematoxylin, and its working solutions show superior stability compared to hematoxylin variants.

Product description

- **ERIOCHROME CYANINE R powder dye** – Powder dye for staining of nuclei

Other materials and reagents used in preparation of the staining solution

- Feric ammonium sulphate, dodecahydrate ($FeNH_4(SO_4)_2 \cdot 12H_2O$)

Preparation of staining solutions

Working solution of ferric eryochrome for progressive staining of nuclei (acc. to Llewellyn):

- Carefully add 10 mL of concentrated hydrochloric acid in 990 mL of distilled/demineralized water and stir well
- Add 0.5 g of Feric ammonium sulphate, dodecahydrate ($FeNH_4(SO_4)_2 \cdot 12H_2O$)
- Stir until completely dissolved
- Add 1 g of Eriochrome Cyanine R powder dye and stir until completely dissolved
- Filtration is not necessary and the solution is ready to use

Sodium acetate, 0.5% solution

- Add 5 g of sodium acetate, anhydrous (or 8.3 g of sodium acetate, trihydrate) to 1000 mL of distilled/demineralized water
- Filtration is not necessary
- Store the solution at +4°C to +5°C or add a single thymol crystal to extend the shelf-life of the solution

Staining procedure – Eriochrome-Eosin, progressive method:

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.	Staining with the working solution of ferric erichrome for progressive staining of nuclei	5-8 minutes
6.	Rinse under tap water	3-5 min
7.	Turn the nuclei blue using 0.5% sodium acetate solution	3-5 min
8.	Rinse with distilled (demi) water	
9.	Stain with one of the eosin counterstains until the section is optimally stained	15 seconds - 2 minutes
	Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for eosin aqueous solutions is 90 seconds to 2 minutes.	
10.	Rinse under tap water	2 min
11.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
12.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
13.	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 VitroGnost cover glass.

Result

Nucleus - blue
Cytoplasm, collagen, muscle fibers, erythrocytes - hues of pink

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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 Eriochrome Cyanine R 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 exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Conn, J. (1977): Biological Stains, 9th ed., Baltimore: Williams and Wilkins Co.
2. Gurr, E. (1971): Synthetic dyes in biology, medicine and chemistry, London: Academic Press

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	Refer to the supplied documentation		Storage temperature range		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						