ERIOGNOST REAGENT

IVD *In vitro* diagnostic medical device CE

Acid fast reagent for nuclear staining, comparable to Hematoxylin reagents
INSTRUCTIONS FOR USE

REF Product code: ERS-OT-100 mL (100 mL) ERS-OT-500 mL (500 mL) ERS-OT-1L (1000 mL)

Introduction

Eriochrome Cyanine R belongs to anionic sulfonphthalein mordant dyes. It can be used independently as pH indicator or as red anionic dye; however, it creates intensely stained complexes with transition metal ions (such as iron ions), and because of that this dye is most commonly used in histology as hematoxylin substitute. Eriochrome represents economic and ecologically acceptable synthetic replacement for hematoxylin, and its working solutions show superior stability compared to hematoxylin working solutions. A mix of Eriochrome Cyanine R and ferrous mordant (Fe) is used for staining. Eriochrome and ferrous ions create 4 different complexes, two of which are red, and two are blue. If these dyes' solutions with ferrous salts are used under suitable conditions, selective nuclear staining may be achieved, similar to hematoxylin staining results. This method is compatible with the usual eosin counterstaining as a substitution for HE staining, but it is also used in special staining kits, such as: A.F.O.G., Gomori Trichrome, Masson-Goldner Trichrome, Masson Trichrome, Picro Sirius Red, Safranin O and Mucicarmine.

Product description

- **ERIOGNOST REAGENT -** Reagent for nuclear histopathology staining. Contains ferrous mordant and Eriochrome Cyanine R powder dye

Other sections and reagents that may be used for this staining method:

- 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, BioWax 56/68, BioWax Blue, BioWax Micro
- 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 C, or BioMount Aqua
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- Counterstaining reagents, such as BioGnost's eosin solutions
- Counterstaining reagents (found in BioGnost's special staining kits)

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), 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, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so that it completely covers the section.

Histology sections staining procedure:

a) combined with eosin solution (alternative to H-E staining)

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 with ErioGnost reagent	5 min
6.	Rinse under tap water until the dye is no longer released from the section	2 exchanges, 15 seconds each
7.	If alcoholic eosin solution is used, immerse the sections in 95% alcohol (Histanol 95). If aqueous eosin solution is used, skip this step.	
8.	Stain using an eosin counterstain solution until section is stained optimally	15 seconds - 2 min
	Note: Intensive eosinophilic dye is achieved much quicker by staining the sections in alcoholic eosin solutions (within 15 seconds); sections should be exposed to aqueous eosin solutions for 90 seconds to 2 minutes	
9.	Rinse under tap water Note: If alcoholic eosin solution is used as counterstain, skip this step.	2 min
10.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips each
11.	Dehydrate u 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips each
12.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each
13.	See Note - covering	

b) dichromatic staining with ErioGnost reagent

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 with ErioGnost reagent	5 min
6.	Rinse with distilled/demi water	2 exchanges, 15 seconds each
	Note: distilled/demi water's pH must be in 5.0-5.5 range	
7.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips each

8.	Dehydrate u 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips each
9.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each
10.	See Note - covering	

c) using special staining kits' reagents as Hematoxylin reagent substitutes

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.	Dip in or drop ErioGnost reagent	5-15 min
	Note: incubate the sections for 15 min in ErioGnost reagent to achieve stronger nuclear staining	
6.	Rinse under tap water	3-10 min
	Note: rinsing period is defined through special staining kit	
7.	Stain with counterstain reagent (depends on the type of special staining dyes used)	
8.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
9.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
10.	Dehydrate u 100% alcohol (Histanol 100)	2 min
11.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each
12.	See Note - covering	

Note - covering

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

Staining procedure / Type of tissue or cell part	**A** - in combination with eosin solution (alternative to H-E staining)	**B** - dichromatic staining with ErioGnost reagent	**C** - with special staining kits' reagents as replacement for hematoxylin reagent
Nuclei	blue	blue	brown to blue
Cytoplasm, collagen, muscle fibers, erythrocytes	hues of pink	hues of pink	depending on the type of counterstaining solutions; see the special staining kit's instructions for use

Note

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 ErioGnost reagent in a tightly sealed original packaging at temperature of +15°C and +25°C. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Clark G. (1979): Staining With Chromoxane Cyanine R, Stain Technol. 54.337-344.
2. Chapman, D. M. (1977): Eriochrome cyanine as a substitute for hematoxylin and eosin, Canad. J. Med.
3. Kiernan, J.A. (1984): Chromoxane cyanine R. I. Physical and chemical properties of the dye and of some of its iron complexes, J. *Microsc.* 134, 13-23.

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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	CE
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					