BIOGNOST®

EOSIN CONTRAST PLUS

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Modified alcoholic solution for more intense and vibrant cytoplasmatic counterstaining

Contains Eosin Y, Phloxine B, and Biebrich Scarlet for additional effect of cytoplasmic counterstaining

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
EOYKP-OT-1L	1000 mL	03858888823429
EOYKP-OT-2.5L	2500 mL	03858888823436

Intended use and test principle

BioGnost's Eosin Contrast Plus is a reagent which is commonly used as a contrast dye for hematoxylin in the histological staining method, the hematoxylin and eosin (HE) staining. This method is used to achieve better visualization and differentiation of cellular structures: the nuclei of microscopic samples are first stained blue with hematoxylin, followed by staining the cytoplasm with eosin in several shades of pink and red. Unlike standard aqueous eosin solutions, alcoholic solution of Eosin Y, Phloxine B, and Biebrich Scarlet (modification of Meter's Eosin) stain slides more intensely pink and red. Eosin Y, Phloxine B, and Blebrich Scarlett are anion dyes that stain erythrocytes bright red, and it also stains basic cellular components, such as cytoplasm, collagen, and muscle fibers.

Product description

- **EOSIN CONTRAST PLUS** – stabilized alcoholic solution of Eosin Y, Phloxine B, and Biebrich Scarlet for more intensive and vibrant cytoplasmic counterstaining.

Additional reagents and materials that can be used in this 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade
- Nuclei bluing reagents such as BioGnost's hematoxylin solutions: Hematoxylin (H, ML, G1, G2, G3 and Hematoxylin M)
- Differentiation reagents such as BioGnost's Acid alcohol
- Bluing reagents such as BioGnost's Bluing reagenst or Scott's solution

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 μm thin slices and mount on a VitroGnost microscope slide

NOTE

Make sure that the part of the slide with the sample is fully immersed in the appropriate solution or reagent at every step of the procedure.

Hematoxylin and eosin (HE) manual* staining procedure, progressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain using Hematoxylin M, Hematoxylin ML, Hematoxylin G1, G2 or Hematoxylin H	3-5 min
	Note: if sedimentation occurs or a metallic sheen forms in hematoxylin solution, filter the reagent before use	
6.	Immerse in distilled/demineralized water until dye is no longer being released from the slide	
7.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Finish the process of bluing after the nuclei turn blue. If no Scott's solution or Bluing reagent is available, rinse the slides under tap water for 3-5 minutes	
8.	Immerse in distilled/demineralized water	
9.	Immerse in 95% alcohol (Histanol 95)	30 sec
10.	Stain using Eosin Contrast PLUS solution	up to 15 sec
11.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
12.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
13.	Clear in xylene (BioClear) or 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.

***** Automatic hematoxylin – eosin (HE) staining method is available in BioGnost's Hem Diff, Hem Diff Strong and BioBluing Buffer instructions for use.

Hematoxylin and eosin (HE) manual* staining procedure, regressive

1.	Deparaffinize in xylene (BioClear) or a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain using Hematoxylin ML, Hematoxylin G3 or Hematoxylin H	4-8 min

	Note: if sedimentation occurs or a metallic sheen forms in hematoxylin solution, filter the reagent before use	
6.	Immerse in distilled/demineralized water until dye is no longer being released from the slide	
7.	Differentiate using Acid alcohol	3-10 dips
	Note: This step removes excessive hematoxylin from the nucleus and cytoplasm. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long	
8.	Rinse in distilled/demineralized water	
9.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Finish the process of bluing after the nuclei turn blue. If no Scott's solution or Bluing reagent is available, rinse the slides under tap water for 3-5 minutes	
10.	Immerse in distilled/demineralized water	
11.	Immerse in 95% alcohol (Histanol 95)	30 sec
12.	Stain using Eosin Contrast PLUS solution	up to 15 sec
13.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
14.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
15.	Clear in xylene (BioClear) or 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.

***** Automatic hematoxylin – eosin (HE) staining method is available in BioGnost's Hem Diff, Hem Diff Strong and BioBluing Buffer instructions for use.

Result

Nuclei - blue

Cytoplasm, collagen, muscle fibers, erythrocytes – hues of pink

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product label.

References

1. Bruce-Gregorios, J.H. (1974): Histopathologic Techniques, *IMC Press Inc.,* Quezon City, Philippines.
2. Cook, D.J. (2009): *Cellular Pathology: An introduction to techniques and applications.* 2nd ed., Scion Publishing Ltd., Bloxham.
3. Gurr, E. (1971): *Synthetic dyes in biology, medicine and chemistry*. Academic Press, London.

Warnings and precautions regarding the materials contained in the product:

H226	Flammable liquid and vapor.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

EOYKP-IFU_ENV5, 19.02.2026., IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
5	Revised in acc. to Regulation (EU) 2017/746 - IVDR	19.02.2026.