BIOGNOST®

BLUING REAGENT

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

Reagent for the conversion of red hematoxylin-stained nuclei to blue

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30799PROCYU	
EMDN code	W01030799	
REF **Catalog number**	**Volume**	**UDI-DI**
BR-OT-500	500 mL	03858892124857
BR-OT-1L	1000 mL	03858888823504
BR-OT-2.5L	2500 mL	03858888823511

Intended use and test principle

Bluing reagent is an aqueous reagent that, thanks to a slightly alkaline pH value in the standard hematoxylin-eosin (HE) staining procedure, replaces tap water during the rinsing step and enables fast and accurate bluing of chromatin in the nucleus and membrane of the cell nucleus. After staining with hematoxylin, the nuclei are initially reddish because the hematoxylin solution is acidic. The bluing reagent is a slightly alkaline solution that increases pH and thus changes the color of the hematoxylin to its blue shape, sharpens the display of the nuclei, increases the contrast of the slide and stabilizes the staining so that the hematoxylin from the nuclei is not easily washed off in the next staining steps. After the action of the bluing reagent, the cell nuclei appear dark blue to blue-violet, and the cytoplasm is later stained with eosin in tones of pinkish red.

Product description

- **BLUING REAGENT** – Lithium carbonate based aqueous reagent for bluing red coloured cores

Example of the use of Bluing reagents during regressive HE staining method

Additional reagents and materials that can be used in the method

- Fixatives, such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Reagents for staining nuclei such as BioGnost's Hematoxylin solutions: Hematoxylin H or Hematoxylin G3
- Contrast staining reagents such as BioGnost's Eosin solutions
- Reagent for differentiation like BioGnost's Acid alcohol

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 micron thin slices and mount on a VitroGnost microscope slide

Hematoxylin-eosin (HE) manual staining procedure, regressive

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 with Hematoxylin H or Hematoxylin G3	4-8 min
	Remark: If there has been precipitation in the solution or the formation of a metallic sheen on the surface, the reagent must be filtered before use	
6.	Immerse the slide in distilled/demineralized water until the release of dye from the slide stops	
7.	Differentiation with Acid alcohol	3-10 dips
	Note: This step removes excess hematoxylin from the nucleus and cytoplasm. If the sample has been treated with a differentiating agent for too long, the nuclei may become discolored	
8.	Rinse in distilled/demineralized water	
9.	Make nuclei turn blue using the Bluing reagent	1 min
	Note: Stop bluing after the nuclei turn blue	
10.	Immerse the slide in distilled/demineralized water	
11.	If an alcoholic solution of eosin is used, immerse the slide in 95% alcohol (Histanol 95). If an aqueous solution of eosin is used, skip this step	
12.	Stain with one of the eosin contrast solutions until the slide is optimally stained	15 s to 2 min
	Note: Staining slides in alcoholic solutions of eosin produces an intense eosinophilic color much faster (within 15 seconds) while exposure to aqueous solutions of eosin is recommended for 90 seconds to 2 minutes.	

13.	Rinse under running tap water Note: If an alcoholic eosin solution is used as a counterstain, skip this step.	
14.	Dehydrate in 95% alcohol (Histanol 95)	2 changes with 10-15 dips
15.	Dehydrate in 100% alcohol (Histanol 100)	3 changes with 10-15 dips
16.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 minutes each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

Result
Nuclei - blue
Cytoplasm, collagen, muscle fibers, erythrocytes - shades of pink

Limitations
This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in BioGnost's instructions for use may cause variations in the 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.
If a serious incident occurs during use 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, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use, 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 production date and expiration date are printed on the product label.

References

1. Sheehan, DC and Hrapchak, BB (1980): Theory and Practice Histotechnology, 2nd ed St. Louise: CV Mosby Co.
2. Kiernan JA (2008) Histological and histochemical methods, 4th ed. Bloxham: Scion Publishing Ltd.

Warnings and precautions regarding the materials contained in the product:
Not a dangerous substance or mixture according to Regulation (EC) no. 1272/2008.

BR-IFU, ENV6, 09.04.2026, 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		Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
6	Revised acc. to Regulation (EU) 2017/746 - IVDR	09.04.2026.