BIOGNOST®

HEM DIFF

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

Differentiation reagent in HE modified regressive staining method

INSTRUCTION FOR USE

BASIC UDI number	385889212HPC30799PROCYU	
EMDN code	W01030799	
REF **Catalogue number**	**Volume**	**UDI-DI number**
HD-OT-1L	1000 mL	03858888829698
HD-OT-2.5L	2500 mL	03858888829759

Intended use and test principle

BioGnost's Hem Diff is a weak differentiation reagent used in modified regressive hematoxylin-eosin (HE) staining (using Hematoxylin G2, G3, ML), in both automated and manual staining procedures. Modified regressive staining refers to a method that includes overstaining of structures and subsequent removal of excess dye from the cytoplasm, differentiation of nuclei with a weaker differentiator, and better binding of eosin. The use of a weak differentiator in modified regressive staining is also necessary due to the removal of excess blue dye from the slides, a phenomenon that occurs mostly due to the vertical position of the slides during HE staining in automatic stainers where the specimens are immersed in a reagent container. A weaker differentiation reagent enables longer incubation and more precise differentiation. Regardless of the fixative in which the tissue is fixed (formalin or glyoxal), Hem Diff gives clear results. The time required for differentiation with the Hem Diff reagent is the same regardless of the fixative used to fix the examined sample.

Description of the product

- **HEM DIFF** - Weak reagent (weak acid solution) for hematoxylin differentiation (pH 3.8) used in modified regressive HE staining method in histology

Additional reagents and materials that can be used in staining

- Fixative agents 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
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost reagents for HE staining such as Hematoxylin G2, G3, ML, and eosin (aqueous and alcoholic)
- A bluing agent such as BioGnost's BioBluing Buffer, Scott's solution or Bluing reagent
- 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

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 sections and mount on a VitroGnost microscope slide

Automated and manual HE staining procedures, modified regressive (examples of staining protocol using Hematoxylin G2, G3, ML)

Notes: If precipitation or the formation of a metallic sheen occurred on the surface of the hematoxylin reagent, the reagent must be filtered before use.

Use a progressive protocol for staining with Hematoxylin G1 and M, a modified regressive protocol for Hematoxylin G2, G3, and ML. If another type of hematoxylin or hematoxylin from another manufacturer is used, the protocols should be optimized.

a) Automated HE staining procedure, modified regressive

STEP	REAGENT	TIME (min)	ACCURACY	LEACHING TIME (sec)	SHAKING
1.	Oven (60 °C)	0:10:00	/	/	/
2.	Deparaffinization in xylene substitute (BioClear New)	0:10:00	NO	10	0
3.	Deparaffinization in xylene substitute (BioClear New)	0:10:00	NO	10	0
4.	Deparaffinization in xylene substitute (BioClear New)	0:10:00	NO	10	0
5.	Rehydrate in 100% alcohol (Histanol 100)	0:03:00	NO	10	0
6.	Rehydrate in 100% alcohol (Histanol 100)	0:02:00	NO	10	0
7.	Rehydrate in 95% alcohol (Histanol 95)	0:02:00	NO	10	0
8.	Rehydration in 70% alcohol (Histanol 70)	0:02:00	NO	10	0
9.	Rinse under running tap water	0:02:00	NO	10	0
10.	Rehydration in distilled/demineralized water	0:01:00	NO	10	0
11.	Staining with Hematoxylin G2/G3/ML	0:04:00/0:02:00/0:06:00	YES	10	0
12.	Rinse under running tap water	0:01:00	YES	10	0
13.	**Differentiation with Hem Diff reagent**	**0:00:20 (G2,ML)/0:01:00 (G3)**	YES	0	0
14.	Rinse under running tap water	0:01:00	NO	10	0
15.	Bluing of nuclei with BioBluing buffer	0:03:00	NO	10	0
16.	Rinse under running tap water	0:01:00	NO	10	0
17.	Dehydrate in 95% alcohol (Histanol 95)	0:00:30	NO	10	0
18.	Staining with Eosin 1% alcohol	0:01:00	YES	10	0
19.	Dehydrate in 95% alcohol (Histanol 95)	0:00:30	YES	10	0
20.	Dehydrate in 95% alcohol (Histanol 95)	0:00:30	YES	10	0
21.	Dehydrate in 100% alcohol (Histanol 100)	0:01:00	YES	10	0
22.	Dehydrate in 100% alcohol (Histanol 100)	0:01:00	YES	10	0
23	Clearing in xylene substitute (BioClear New)	0:05:00	NO	10	0
24	Clearing in xylene substitute (BioClear New)	0:05:00	NO	10	0
25	Clearing in xylene substitute (BioClear New)	0:05:00	NO	10	0

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.

b) Manual HE staining procedure, modified regressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	2 changes for 3 minutes (BioClear) or 3 changes for 10 minutes (BioClear New)
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 2 minutes each
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydration in 70% alcohol (Histanol 70)	2 minutes
5.	Rinse under running tap water	2 minutes

6.	Rehydrate in distilled/demineralized water	1 minute
7.	Staining with Hematoxylin G2/G3/ML	4 minutes (G2), 2 minutes (G3) or 6 minutes (ML)
8.	Rinse under running tap water	1 minute
9.	**Differentiation with Hem Diff reagent**	**20 seconds (G2, ML) or 1 minute (G3)**
10.	Rinse under running tap water	1 minute
11.	Bluing of nuclei with BioBluing buffer	3 minutes
12.	Rinse under running tap water	1 minute
13.	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	30 seconds
14.	Staining with Eosin 1% alcohol or Eosin 1% aqueous	1 minute (alcohol eosin) or 2 minutes (aqueous eosin)
	Note: If hematoxylin G3 is used, use Eosin 2% alcoholic or 2% aqueous for contrast staining.	2 minutes
15.	Rinse under running tap water	15 seconds
	Note: If an alcoholic solution of eosin is used as a contrast stain, skip this step.	
16.	Dehydrate in 95% alcohol (Histanol 95)	2 changes lasting 30 seconds each
17.	Dehydrate in 100% alcohol (Histanol 100)	2 changes lasting 1 minute each
18.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 changes for 3 minutes (BioClear) or 3 changes for 10 minutes (BioClear New)

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 - dark blue color
Cytoplasm, collagen, elastin, erythrocytes – shades of pink
Goblet cells (mucins), cartilage matrix – blue-purple color

Picture 1. Human skin stained with Hematoxylin G2 and Eosin 1% alcoholic (modified regressive staining).

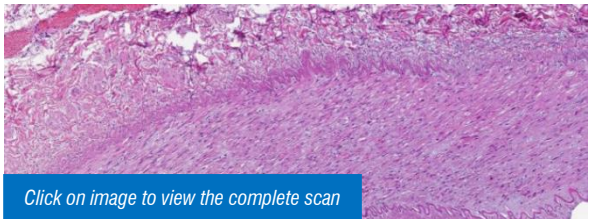

Figure 2. Human artery stained with Hematoxylin G2 and Eosin 1% alcoholic (modified regressive staining).

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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, histological processing of samples 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.

Literature

1. Bancroft JD, Gamble M: Theory and Practice of Histological Techniques. 6th Ed., Churchill-Livingstone Elsevier, Nottingham, UK, 2008.
2. Carson FL, Hladik C: Histotechnology A Self-Instructional Text. 3rd Ed., *ASCP Press*, Dallas, TX, 2009.
3. Guidelines for hematoxylin & eosin staining: National Society for Histotechnology

Warnings and precautions regarding the materials contained in the product:	
	H225 Highly flammable liquid and vapour. 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.

HD-IFU_EN1, 11.07.2024, SB/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		

BioGnost Ltd.
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
1.	The first version of the instructions for use	11.07.2024.