BIOGNOST®

HEMATOXYLIN INSTANT KIT

IVD *In vitro* diagnostic medical device CE

For fast and simple preparation of 1 liter of Hematoxylin Instant, used in histology and cytology

Contains two components

INSTRUCTIONS FOR USE

REF Catalogue number: HEMI-K-1L (for preparing 1 L of Hematoxylin Instant) HEMI-K-6L (for preparing 6 x 1 L of Hematoxylin Instant)

Introduction:

BioGnost's Hematoxylin Instant kit is specially prepared for fast and practical preparation of Hematoxylin Instant that can be used for staining histology and cytology samples. It contains two specially stabilized components that (by dissolving in distilled water) create a formulation of hematoxylin that may be used as a substitute for Harris' or Gill's hematoxylin. Hematoxylin Instant provides very precise and clear results of cellular nuclei, and may also be used in progressive and in regressive staining methods. It also does not contain oxidant based on mercury chloride. That makes it environment-friendly.

Product description:

- **HEMATOXYLIN INSTANT KIT** – kit for preparing Hematoxylin Instant for nuclear staining in histopathology and cytology.

Hematoxylin Instant kit contains:	for preparing 1 L of Hematoxylin Instant (HEMI-K-1L)	for preparing 6 x 1 L of Hematoxylin Instant (HEMI-K-6L)
Hematoxylin Instant - component A	1 x HEMI-KA	6 x HEMI-KA
Hematoxylin Instant - component B	1 x HEMI-KB	6 x HEMI-KB

Preparing Hematoxylin Instant

1.	Pour all the contents of components A and B of Hematoxylin Instant in the container that can fit more than 1 L of fluid.
2.	Add 1 L of distilled (demi) water. DO NOT USE TAP WATER. If Hematoxylin Instant is used for staining cytology sections or in progressive staining methods, dissolve components A and B in 1.5 do 2 L of distilled (demi) water.
	Note: in order to achieve better stability, less precipitate and longer expiry date, we recommend 900 mL of distilled water and 100 mL of ethylene glycol (CAS: 107-21-1). For cytology or progressive staining - dissolve the components in 1350 mL of distilled water and 150 mL of ethylene glycol or, for even more specific staining, in 1800 mL of distilled water and 200 mL of ethylene glycol.
3.	Mix the solution well until the contents melt. Wait for at least 60 minutes before first staining.
	Note: for optimal results wait 12 hours before first staining.
4.	Filter Hematoxylin Instant before use. Precipitate and metallic surface are normal occurrence for this type of product.
5.	Keep Hematoxylin Instant 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. Shelf life of prepared Hematoxylin Instant is 6-9 months.

Using Hematoxylin Instant:

a) in histological sections staining procedure

Preparing histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), 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 56/58, BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

Hematoxylin and eosin (HE) staining procedure, regressive

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in descending series of alcohols (Histanol 100, Histanol 95) and in distilled (demi) water	10 dips in each of the 3 exchanges
3.	Stain using Hematoxylin Instant	2-5 minutes
	Note: incubation period is shorter with progressive staining	30 seconds to 2 min
4.	Rinse with distilled (demi) water	1 min
5.	Differentiate in Acid alcohol	3-10 fast dips
	Note: This step removes excessive hematoxylin. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long. Skip this step with progressive staining	
6.	Rinse in distilled (demi) water	
7.	Blue using Scott's solution or Bluing reagent	1 min
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
8.	Use Eosin Instant, alcoholic or aquoues until the section is optimally stained. Eosin Instant, alcoholic will achieve optimal staining for 30 seconds - 1 minute, and Eosin Instant, aqueous in under 3 minutes.	30 seconds - 3 min
	Note: Eosin Instant may be substituted with any BioGnost's aqueous or alcoholic Eosin solution	
9.	Rinse under tap water	30 seconds
10.	Dehydrate using 95% alcohol (Histanol 95)	10-15 dips in each of the 2 exchanges
11.	Completely rehydrate by using 100% alcohol (Histanol 100)	10-15 dips in each of the 3 exchanges
12.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 minutes in each of the 2 exchanges

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 a VitroGnost cover glass.

Result:
Nuclei - blue
Cytoplasm, collagen, muscle fibers, erythrocytes - hues of pink to red

b) in cytology (and gynecology samples) staining methods acc. to Papanicolaou, <u>regressive</u>

The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide.

If the sample is dry and previously fixated by using solution containing polyglycols (CitoSpray), it is necessary to keep it in a 95% alcohol solution (Histanol 95) for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution (Histanol 95), ignore this step. During staining cytology samples (prepared by using the liquid based cytology method (LBC)) that contain low concentration of alcohol, rehydration by descending series of alcohol solutions is not necessary. The procedure begins by rinsing the section using distilled (demi) water and is then stained using Hematoxylin Instant.

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 80 and Histanol 70) and in distilled or demineralized water	6-8 dips in each of the 4 exchanges
2.	Stain using Hematoxylin Instant	4 min
	Note: incubation period is shorter with progressive staining	30 sec
3.	Rinse in distilled/demineralized water	6-8 dips
4.	Differentiation using HCL Pap reagent or in 0.1% HCl solution	5-10 seconds
	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.	
5.	Rinse in distilled water	6-8 dips
6.	Blue using Scott's solution or Bluing reagent	1 min
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
7.	Dehydrate in ascending series of alcohols (Histanol 70, Histanol 80 and Histanol 95)	6-8 dips in each of the 3 exchanges
8.	Stain using OG-6, Pap 2A reagent	2 min
9.	Rinse using 95% alcohol in two exchanges (Histanol 95)	6-8 dips in each of the 2 exchanges
10.	Stain using EA 31, Pap 3A reagent or EA 50, Pap 3B reagent	4 min
11.	Rinse using 95% alcohol (Histanol 95)	6-8 dips
12.	Dehydrate using 100% alcohol (Histanol 100)	6-8 dips
13.	Dehydrate using 100% alcohol (Histanol 100)	3-5 minutes
14.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	6-8 dips
15.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3-5 minutes

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 a VitroGnost cover glass.

Results
Nuclei - blue
Keratinized cells - yellow-orange
Superficial squamous epithelial cell, erythrocytes, nucleoli, cilia - pink-red
Cytoplasm of all the other cell types (parabasal and intermediate squamous cells, columnar cells, polymorphonuclear leukocytes, lymphocytes, histiocytes, adenocarcinomas, undifferentiated carcinoma cells) - green

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 Hematoxylin Instant kit 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. Baker, J.R. (1962): Experiments on the action of mordants. 2. Aluminium-hematein. *Q.J.Microsc. Sci.* p103 493-517.
2. Conn, J. (1977): *Biological Stains*, 9th ed., Baltimore: Williams and Wilkens Co.
3. Harris, H.F. (1898): A new method of "ripening" haematoxylin. *Microsc. Bull.* (Philadelphia) Dec. 47.
4. Harris, H.F. (1900): On the rapid conversion of haematoxylin into haematein in staining reactions. J. *Appl. Microsc.* p3 777-780.

HEMI-K-X, V3-EN3, 10 July 2019, AK/IŠP

Refer to the supplied documentation	Storage temperature range	Number of tests in package	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 number	Manufacturer		
For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile				