BIOGNOST®

KIT FOR RAPID HE STAINING OF FROZEN AND PARAFFIN-EMBEDDED SECTIONS

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

Ready-to-Use eight-reagent (in 20 jars) kit for rapid H&E frozen and paraffin sections staining in histopathology, contains medium for permanent section covering

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
HE-RTU-100T	20x70 mL	03858890009996

Intended use and test principle

The Ready-To-Use staining kit for frozen and paraffin-embedded histological sections using the H&E (hematoxylin–eosin) method enables complete sample processing. In the case of frozen sections, the procedure can be completed within just a few minutes after sectioning the sample with a cryostat. The staining procedure is highly simplified and finished in only a few minutes. The kit contains all reagents required for sample processing – from BioFix fixative for cryostat sections and the xylene substitute BioClear New for deparaffinization and clearing of paraffin sections, alcohol solutions for tissue rehydration and dehydration, and deionized water, to hematoxylin and eosin solutions and a bluing reagent for nuclear staining. It also includes a low-viscosity mounting medium with an optimal refractive index, BioMount New, supplied in practical packaging. The reagents are provided in convenient containers into which slides are directly immersed. They are arranged and labeled in the box according to the order of use, reducing the risk of reagent contamination during staining. Any reagents not used during the staining of frozen or paraffin sections can be reused in subsequent staining procedures. The kit includes an additional container of BioFix solution and Bluing reagent, intended for use after 50 stained slides. The kit is sufficient for staining approximately 100 slides..

Product description

- **KIT FOR RAPID HE STAINING OF FROZEN AND PARAFFIN SECTIONS** – Eight-reagent kit (in 20 containers) for rapid HE staining of frozen and paraffin sections in histopathology, contains medium for permanent section covering

The kit contains:	100 tests (HE-RTU-100T)	Storage temperature
BioClear New	6 x 70 mL	15-25°C
BioFix	2 x 70 mL	15-25°C
Histanol 100	3 x 70 mL	15-25°C
Histanol 95	3 x 70 mL	15-25°C
Deionized water	2 x 70 mL	15-25°C
Hematoxylin G2	1 x 70 mL	15-25°C
Bluing reagent	2 x 70 mL	15-25°C
Eosin Y 2% alcoholic	1 x 70 mL	15-25°C
BioMount New Low	2 x 10 mL	15-25°C

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
- Embedding medium for cryostat sectioning in different colors, such as BioGnost's CryoFix Gel

A1) 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: The jars are placed and marked in order of use. Open the jars before staining. Immediately after finishing the staining process, close the jars using screw caps and close tightly in order to prevent evaporation.

Paraffin section staining procedure

Skip the jar 1 and start the staining procedure from jar 2 that contains BioClear News (xylene substitute) Continue staining until the end (jar 18). Change deionized water regularly.

Step	Container Nr.		
1.	**2**	Deparaffinize in xylene substitute (BioClear New)	1 min
2.	**3**	Deparaffinize in xylene substitute (BioClear New)	1 min
3.	**4**	Deparaffinize in xylene substitute (BioClear New)	1 min
4.	**5**	Rehydrate in 100% alcohol (Histanol 100)	10 dips
5.	**6**	Rehydrate in 95% alcohol (Histanol 95)	10 dips
6.	**7**	Rinse in Deionized water	10 dips
7.	**8**	Stain using Hematoxylin reagent	1-3 min
8.	**9**	Rinse in Deionized water	10 dips
9.	**10**	Nuclear bluing with Bluing reagent	1 min
		Note: in order to preserve the solution's alkalinity, after 50 stained sections replace the used Bluing reagent with a new one (contained in the kit)	
10.	**11**	Dehydrate in 95% alcohol (Histanol 95)	10 dips
11.	**12**	Stain using Eosin reagent	5-15 sec
		Note: if the intensity of Eosin reagent is too strong, it can be diluted using 80-85% ethyl alcohol	
12.	**13**	Dehydrate in 95% alcohol (Histanol 95)	10 dips
13.	**14**	Dehydrate in 100% alcohol (Histanol 100)	10 dips
14.	**15**	Dehydrate in 100% alcohol (Histanol 100)	10 dips
15.	**16**	Clear in xylene substitute (BioClear New)	10 dips
16.	**17**	Clear in xylene substitute (BioClear New)	10 dips
17.	**18**	Clear in xylene substitute (BioClear New)	1 min

Immediately after clearing apply an appropriate BioMount New medium for covering/mounting cover glass Cover the section with a VitroGnost cover glass.

Result

Nuclei – blue – purple
Cytoplasm, collagen, elastin, erythrocytes – hues of red – pink

A2) Preparing the frozen section for staining

- Freeze the tissue as quickly as possible in order to avoid forming artefacts and distortion: embed the tissue sample in CryoFix gel and freeze
- Place the frozen block on cryostat and cut the section Optimal cryostat sectioning temperature is -5 to -6°C for samples thicker than 15 μm. Thinner sections require lower temperature.
- Mount the section on the glass slide and immediately start staining procedure (immerse in BioFix - jar 1).

Frozen sections staining procedure

Start the section staining from jar 1 containing BioFix; skip jars 2, 3, 4, 5, and 6. After jar 1 continue staining from jar 7. Continue with staining until the end (jar 18). Change deionized water regularly.

Step	Container Nr.		
1.	**1**	Fixa in BioFix solution	10 sec
		Note: after staining 50 sections, replace BioFix solution with a fresh one (contained in the kit)	
2.	**7**	Rinse in Deionized water	10 dips
3.	**8**	Stain using Hematoxylin reagent	1-3 min
4.	**9**	Rinse in Deionized water	10 dips
5.	**10**	Nuclear bluing with Bluing reagent	1 min
		Note: in order to preserve the solution's alkalinity, after 50 stained sections replace the used Bluing reagent with a new one (contained in the kit)	
6.	**11**	Dehydrate in 95% alcohol (Histanol 95)	10 dips
7.	**12**	Stain using Eosin reagent	5-15 sec
		Note: if the intensity of Eosin reagent is too strong, it can be diluted using 80-85% ethyl alcohol	
8.	**13**	Dehydrate in 95% alcohol (Histanol 95)	10 dips
9.	**14**	Dehydrate in 100% alcohol (Histanol 100)	10 dips
10.	**15**	Dehydrate in 100% alcohol (Histanol 100)	10 dips
11.	**16**	Clear in xylene substitute (BioClear New)	10 dips
12.	**17**	Clear in xylene substitute (BioClear New)	10 dips
13.	**18**	Clear in xylene substitute (BioClear New)	1 min

Immediately after clearing apply an appropriate BioMount New medium for covering/mounting cover glass Cover the section with a VitroGnost cover glass.

Result

Nuclei – blue – purple
Cytoplasm, collagen, elastin, erythrocytes – hues of red – 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. To avoid an incorrect staining result, it is advised to use a positive and negative control.
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. Gill, G.W., Frost, J.K, Miller, K.A. (1974): A new formula for half-oxidized hematoxylin formula that neither overstains nor requires differentiation. Acta Cytol. 1974;18:300-301.
2. Gill, G.W. (2006): Enviro-Pap: an environmental friendly, economical, and effective Pap stain. Lab. Med. 37: str. 105-108.
3. Papanicolaou, G.N. (1954): A new procedure for staining vaginal smears. Science. 95: str. 438-439.
4. Sheehan, D.C. i Hrapchak, B.B. (1980): Theory and Practice of Histotechnology, 2nd ed., St. Louise: CV Mosby Co

Warnings and precautions regarding the materials contained in the product:

EUH066	Repeated exposure may cause skin dryness or cracking.
H225	Highly flammable liquid and vapor.
H302	Harmful if swallowed.
H304	May be fatal if swallowed and enters airways.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H370	Causes damage to organs.
H301+H311+H331	Toxic if swallowedm if on skin or if inhaled.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P331	Do NOT induce vomiting.
P370+P378	In case of fire: use CO_2 and fire foam to extinguish
P301+P310	IF SWALLOWED: call immediately POISON CENTER/doctor.
P403+P235	Store in a well- ventilated place. Keep cool.
P308+P311	IF exposed or concerned: call POISON CENTER/doctor.
P301+P312	IF SWALLOWED: call POISON CENTER/doctor if you feel unwell.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

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

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Version	Description / reason for change	Date
5	Correction of Kit Contents	02.06.2026.