BIOGNOST®

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

IVD In vitro diagnostic medical device CE

Ready-To-Use kit for rapid hematoxylin-eosin staining frozen and paraffin sections, contains medium for permanent section covering

INSTRUCTIONS FOR USE

REF Catalogue number: HE-RTU-100T (for 100 tests)

Introduction

Ready-To-Use kit for staining frozen and paraffin histology sections using HE (hematoxylin-eosin) method enables complete tissue processing. In case of processing frozen sections, it is completed in just a few minutes after sectioning the sample using cryotome. The staining procedure is simplified and conducted in a few minutes. The kit contains all the reagents necessary for sample processing - BioFix medium for fixing cryostat samples, BioClear New (xylene substitute) for deparaffnation and clearing paraffin sections, alcoholic solutions for rehydration and dehydration of tissue, deionized water, as well as hematoxylin and eosin, and nuclear bluing medium. It also contains mounting medium of very low viscosity and optimal refractive index (BioMount New) in practical packaging. The reagents are placed in practical jars and sections may be directly immersed. They are placed in the order of use in the box, which lowers the possibility of contamination of reagents during staining. Reagents that have not been used during staining frozen or paraffin sections may be additionally used in the following staining procedures. The kit contains one additional jar of BioFix solution used after staining 50 sections. The kit is sufficient for staining approximately 100 sections.

Product description

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

The kit contains:	Amount and volume
BioClear New	6 x 70 mL
BioFix	2 x 70 mL
Histanol 100	3 x 70 mL
Histanol 95	3 x 70 mL
Deionized water	2 x 70 mL
Hematoxylin G2	1 x 70 mL
Bluing reagent	2 x 70 mL
Eosin Y 2% alcoholic	1 x 70 mL
BioMount New Low	2 x 10 mL

Other slides and reagents that may be used in staining

- Fixative such as BioGnost's neutral buffered formalin: Formaldehyde NB 4%, Formaldehyde NB 10%
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax 52/54, BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro.
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- CryoFix Gel, medium for embedding tissue samples for cryostat sectioning

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.

A1) Preparing the paraffin section for staining

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

Paraffin section staining procedure

Skip the jar 1 and start the staining procedure from jar 2 that contains BioClear News (xylene substitute) Continue with staining until the end (jar 18)
Change deionized water regularly; change alcohols and BioClear New (xylene substitute) if necessary

Step	Jar		
1.	**2**	Deparaffinize the section in xylene substitute (BioClear New)	1 min
2.	**3**	Deparaffinize the section in xylene substitute (BioClear New)	1 min
3.	**4**	Deparaffinize the section in xylene substitute (BioClear New)	1 min
4.	**5**	Rehydrate using 100% alcohol (Histanol 100)	10 dips
5.	**6**	Rehydrate using 95% alcohol (Histanol 95)	10 dips
6.	**7**	Rinse in deionized water	10 dips
7.	**8**	Stain using Hematoxylin reagent	1-3 minutes
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 using 95% alcohol (Histanol 95)	10 dips
11.	**12**	Stain using Eosin reagent	5-15 seconds
		Note: if the intensity of Eosin reagent is too strong, it can be diluted using 80-85% ethyl alcohol	
12.	**13**	Dehydrate using 95% alcohol (Histanol 95)	10 dips

13.	**14**	Dehydrate using 100% alcohol (Histanol 100)	10 dips
14.	**15**	Dehydrate using 100% alcohol (Histanol 100)	10 dips
15.	**16**	Clear the section in xylene substitute (BioClear New)	10 dips
16.	**17**	Clear the section in xylene substitute (BioClear New)	10 dips
17.	**18**	Clear the section 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.

Results

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 section staining procedure

Start the staining section 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; change alcohols and BioClear New (xylene substitute) if necessary

Step	Jar		
1.	**1**	Fix in BioFix solution	10 seconds
		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 minutes
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 using 95% alcohol (Histanol 95)	10 dips
7.	**12**	Stain using Eosin reagent	5-15 seconds
		Note: if the intensity of Eosin reagent is too strong, it can be diluted using 80-85% ethyl alcohol	
8.	**13**	Dehydrate using 95% alcohol (Histanol 95)	10 dips
9.	**14**	Dehydrate using 100% alcohol (Histanol 100)	10 dips
10.	**15**	Dehydrate using 100% alcohol (Histanol 100)	10 dips
11.	**16**	Clear the section in xylene substitute (BioClear New)	10 dips
12.	**17**	Clear the section in xylene substitute (BioClear New)	10 dips
13.	**18**	Clear the section 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.

Results

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

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 the kit in a tightly sealed original packaging 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. 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.* p37 105-108.
3. Papanicolaou, G.N. (1954): A new procedure for staining vaginal smears. *Science.* p95 438-439.
4. Sheehan, D.C. et Hrapchak, B.B. (1980): *Theory and* Practice *of Histotechnology*, 2nd ed., St. Louise: CV Mosby Co

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