BIOGNOST®

H.B.F.P. KIT

IVD *In vitro* diagnostic medical device CE

Three-reagent kit for staining histological sections of myocardial infarction
Hematoxylin - Basic Fuchsin - Picric Acid

INSTRUCTIONS FOR USE

REF Catalogue number: HBFP-100T (3x30 mL) HBFP-K-100 (3x100 mL)

Introduction

Histological diagnosis of ischemia in early phase or myocardial infarction using the standard hematoxylin-eosin histological methods and light microscope is exceptionally delicate. The reason for that are minimal histopathological changes occurring on the cardiac muscle during the first six hours of symptoms.
However, staining the section using the kit consisting of hematoxylin, basic fuchsin and picric acid enables a histological overview of early changes on the cardiac muscle caused by ischemia or myocardial infarction.

Product description

- **H.B.F.P. KIT** – Three-reagent kit for determining ischemia or myocardial infarction.

The kit contains:	100 tests (HBFP-100T)	3 x 100 mL (HBFP-K-100)
Hematoxylin ML	30 mL (HEMML-OT-30)	100 mL (HEMML-OT-100)
Fuchsin Basic, solution	30 mL (FBO-OT-30)	100 mL (FBO-OT-100)
Picric acid in acetone, solution	30 mL (PKA-OT-30)	100 mL (PKA-OT-100)

Other sections and reagents that may be used in staining:

- 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 agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing the histological sections for staining

- Fixate 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 Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests (HBFP-100T)

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Stain using Hematoxylin ML (add ≥5 drops)	5 min
6.	Rinse under tap water	3 min
7.	Stain with Fuchsin Basic solution (add ≥5 drops)	3 min
8.	Rinse in distilled (demi) water	
9.	Wash the section in absolute alcohol (Histanol 100)	
10.	Differentiate the section using Picric acid solution in acetone (add ≥5 drops)	15-20 seconds
	Note: It is important to treat the microscopical sections individually and carefully using fresh uncontaminated regents during the phase of differentiation by picric acid solution in acetone. Differentiation is finished when erythrocytes, collagen tissue and the ischemic muscle remain crimson red, and the rest of the tissues remain yellow. If these parameters are not carefully monitored, a false or positive or false negative appearance of the section might occur. In that case, the most sensitive criterium is decolorization of erythrocytes.	
11.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 2 min each
12.	Wash the section in absolute acetone	
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

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.

b) using three-reagent 100 mL kit (HBFP-K-100)

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Immerse into Hematoxylin ML	5 min
6.	Rinse under tap water	3 min
7.	Immerse into Fuchsin Basic solution	3 min
8.	Rinse in distilled (demi) water	
9.	Wash the section in absolute alcohol (Histanol 100)	
10.	Differentiate the section by immersing it into picric acid in acetone solution	15-20 seconds
	Note: It is important to treat the microscopical sections individually and carefully using fresh uncontaminated regents during the phase of differentiation by picric acid solution in acetone. Differentiation is finished when erythrocytes, collagen tissue and the ischemic muscle remain crimson red, and the rest of the tissues remain yellow. If these parameters are not carefully monitored, a false or positive or false negative appearance of the section might occur. In that case, the most sensitive criterium is decolorization of erythrocytes.	
11.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 2 min each
12.	Wash the section in absolute acetone	
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

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

Ischemic cardiac muscle, erythrocytes and collagen muscle - crimson red
Nuclei - blue
The rest of the tissues and structures - yellow

Note

Read out the results within a few hours after the staining procedure is finished, since the intensity of the dye fades and probability of erroneous read out rises. Time periods of staining procedures are not standardized. 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 H.B.F.P. 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. Lie JT et al. (1971): New histochemical method for morphologic diagnosis of early stages of myocarddial ischemia. *Mayo Clin Proc*, 46:319-27.
2. HK Al-Rufaie et al. (1983): Comparison of the haematoxylin basic fuchsin picric acid method and the fluorescence of haematoxylin and eosin stained sections for the identification of early myocardial infarction. *J Clin Pathol*, 36: 646-649

HBFP-X, V7-EN3, 14 February 2017, AK/VR

Refer to the supplied documentation	Refer to supplied instructions	For *in vitro* diagnostic use only	Storage temperature range	Keep away from heat and sunlight	Keep in dry place	Number of tests in package	Valid until	Caution - fragile	Product code	Lot number	European Conformity	Manufacturer

BIOGNOST Ltd.
Medjugorska 59
10040 Zagreb
CROATIA
www.biognost.com