

H.B.F.P. KIT



IVD In vitro diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

Three-reagent kit for staining histological sections of myocardial infarction Contains Hematoxylin, New Fuchsin and Picric Acid

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF		
EMDN code	W01030708		
REF	Catalogue number	Volume	UDI-DI number
	HBFP-100T	100 tests	03858892126202
	HBFP-K-100	3 x 100 mL	03858892126219



Intended use and test principle

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, new fuchsin (which is much more stable formulation than the classic 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)
New Fuchsin, solution	30 mL (NFO-OT-30)	100 mL (NFO-OT-100)
Picric acid in acetone, solution	30 mL (PKA-OT-30)	100 mL (PKA-OT-100)

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
- 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
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion media such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Absolute acetone (such as BioGnost's Acetone for histology)

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 slices and mount on a VitroGnost microscope slide

NOTE

Apply the reagent to completely cover the section.

Sample staining procedure

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

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain using Hematoxylin ML (apply ≥5 drops)	5 min
6.	Rinse under tap water	3 min
7.	Stain with New Fuchsin, solution (apply ≥5 drops)	3 min
8.	Rinse in distilled/demineralized water	
9.	Wash the section in absolute alcohol (Histanol 100)	
10.	Differentiate the section using Picric acid solution in acetone (apply ≥5 drops)	15-20 seconds
	Note: It is important to treat the microscopical sections individually and carefully using fresh uncontaminated reagents 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 in 100% alcohol (Histanol 100)	3 exchanges, 2 min each
12.	Wash the section in absolute acetone	
13.	Clear in xylene (BioClear) or 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.

* To avoid section fading (and consequently loss of yellow), we recommend clearing in xylene (BioClear) and mounting with BioMount DPX or BioMount DPX New media.

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

Pour the reagents into glass staining jars (Coplín, Hellendahl or Schifferdecker), return to original bottles after staining. Close well. If necessary, filter the reagents.

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min

4.	Rehydrate in distilled/demineralized water	2 min
5.	Immerse in Hematoxylin ML	5 min
6.	Rinse under tap water	3 min
7.	Immerse in New Fuchsin, solution	3 min
8.	Rinse in distilled/demineralized 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 reagents 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 in xylene (BioClear) or 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.

* To avoid section fading (and consequently loss of yellow), we recommend clearing in xylene (BioClear) and mounting with BioMount DPX or BioMount DPX New media.

Result

Ischemic cardiac muscle, erythrocytes and collagen muscle - crimson red

Nuclei - blue

The rest of the tissues and structures - yellow

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 result. 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.

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, mounting of the slides, and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid a false result, it is recommended 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, transport, 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.

References

- Lie JT et al. (1971): New histochemical method for morphologic diagnosis of early stages of myocardial ischemia. *Mayo Clin Proc*, 46:319-27.
- 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

Warnings and precautions regarding the materials contained in the product:	
	<p>H225 Highly flammable liquid and vapor. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. EUH066 Repeated exposure may cause skin dryness or cracking.</p> <p>P102 Keep out of reach of children. P210 Keep away from heat/sparks/open flames/hot surfaces. Do not smoke. 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. P403 Store in a well-ventilated place.</p>

HBFP-IFU_EN6, 09 April, 2025 KB/ISP

 Manufacturer	 Batch code	 Temperature limit	 <i>In vitro</i> diagnostic medical device	 Unique device identifier
 Date of manufacture	 Catalogue number	 Consult Instructions for use	 Contains sufficient for <n> tests	
 Use-by date	 Fragile, handle with care	 Caution	 European conformity	

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Version	Description / reason for change	Date
6.	Addition of absolute acetone to the section: Additional reagents and materials that can be used in staining	09.04.2025.