

PICRIC ACID IN ACETONE, SOLUTION

IVD *In vitro* diagnostic medical device



For use with H.B.F.P. kit

INSTRUCTIONS FOR USE

REF Product code: PKA-OT-100 (100 mL)

Introduction

Picric acid in acetone, solution is a component of H.B.F.P. kit used for displaying early changes on cardiac muscle caused by ischemia or myocardial infarction. 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.

Product description

- **PICRIC ACID IN ACETONE, SOLUTION** - Picric acid in acetone, solution

Example of using Picric acid in acetone, solution with H.B.F.P. kit

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 52/54, 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 New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua
- 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
- Other components of HBFP kit: Hematoxylin ML (HEMML-OT-100) and Fuchsin Basic, solution (FBO-OT-100)

Preparing the histological sections 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 Plus, BioWax 52/54, BioWax 56/58, BioWax Blue).
- 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

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 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 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 VitroGnost cover glass.

*To avoid section fading (loss of yellow) we recommend clearing in xylene (BioClear) and mounting a glass slide using BioMount DPX or BioMount DPX New medium.

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 Picric acid, solution in a tightly sealed original packaging at temperature of 15°C to 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 myocardial 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

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	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				



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

