BIOGNOST®

HEMATOXYLIN P.T.A. KIT

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

Hematoxylin Phosphotungstic Acid kit for differentiating muscle tissues according to Mallory

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
HPTA-100T	For 100 tests	03858890002607
HPTA-K-100	4x100 mL	03858890008340

Intended use and principle

Hematoxylin P.T.A. kit is used for better visualization of elements of central nervous system, fibrins, but primarily for differentiation between smooth and striated muscle tissue according to Mallory. When staining with Hematoxylin P.T.A. kit a redox reaction occurs between potassium permanganate and oxalic acid when sulfuric acid is present. Potassium permanganate acts as a strong oxidizing agent and oxidizes tissue components causing a reduction and creating a brown manganese dioxide precipitate. Oxalic acid then removes that precipitate (preparation bleaching) while the sulfuric acid provides an acidic medium necessary for the reaction. After oxidation and bleaching, staining with Hematoxylin P.T.A. follows. Hematoxylin P.T.A. selectively binds to certain structures (connective tissue and muscle fibers in particular) giving them a distinct coloration.

Product description

- **HEMATOXYLIN P.T.A. KIT** – Kit for staining and differentiating muscle tissue

The kit contains:	100 tests (HPTA-100T)	4 x 100 mL (HPTA-K-100)	Storage temperature
Potassium permanganate, 0.5% solution	30 mL (KP05-OT-30)	100 mL (KP05-OT-100)	15-25°C
Sulfuric acid, 0.5% solution	30 mL (SK05-OT-30)	100 mL (SK05-OT-100)	15-25°C
Oxalic acid, 1% solution	30 mL (OKS1-OT-30)	100 mL (OKS1-OT-100)	15-25°C
Hematoxylin P.T.A.	100 mL (HPTA-OT-100)	100 mL (HPTA-OT-100)	15-25°C

Additional reagents and materials that can be used in the 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

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

Apply the reagent so it completely covers the section.

Histological sample staining procedure

a) using kit for 100 tests (HPTA-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.	Add 5 drops of Potassium permanganate, 0.5% solution and 5 drops of Sulfuric acid, 0.5% solution. Gently stir and let it react.	5 min
6.	Rinse in distilled/demineralized water	
7.	Treat with Oxalic acid, 1% solution (≥ 5 drops)	5 min
8.	Rinse in distilled/demineralized water	
9.	Dip the slide into Hematoxylin P.T.A. Note: if you wish to accelerate the staining process, heat Hematoxylin P.T.A. for 20 seconds in microwave oven (500 W), remove the solution from the oven, immerse the slide and incubate for 15 minutes. Macroscopically check for section coloration; in case of inadequate coloration, repeat the procedure until adequate coloration is achieved	incubate overnight at room temperature
10.	Rinse in distilled/demineralized water	3-4 sec
11.	Differentiate in 95% alcohol	several quick dips
12.	Dehydrate in 100% alcohol (Histanol 100)	2 min
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 slide. 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.

b) using four-reagent kit, 100 mL (HPTA-K-100)

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.	Prepare working solution: mix equal volumes of Potassium permanganate, 0.5% solution and Sulfuric acid, 0.5% solution	5 min
	Note: Always prepare fresh working solution. Prepare sufficient volume for staining all necessary samples.	
6.	Rinse in distilled/demineralized water	
7.	Immerse into Oxalic acid, 1% solution	5 min
8.	Rinse in distilled/demineralized water	
9.	Dip the section into Hematoxylin P.T.A. Note: if you wish to accelerate the staining process, heat Hematoxylin P.T.A. for 20 seconds in microwave oven (500 W), remove the solution from the oven, immerse the section and incubate for 15 minutes. Macroscopically check for section coloration; in case of inadequate coloration, repeat the procedure until adequate coloration is achieved	incubate overnight at room temperature
10.	Rinse in distilled/demineralized water	3-4 sec
11.	Differentiate in 95% alcohol	several quick dips
12.	Dehydrate in 100% alcohol (Histanol 100)	2 min
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 VitroGnost cover glass.

Result

Nuclei, fibrin, myofibrils, astrocytes, certain elastic fibers, myelinated fibers, glial cells – dark blue
Collagen, cartilage, bone matrix – hues of brick red

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. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2 ed ed., Butterworth, London, UK.
2. Lillie, R.D. (1945): Studies on selective staining of collagen with acid aniline dyes, J. Technical Methods, 25:1
3. Peers, J.H. (1941): A modification of Mallory's Phosphotungstic acid hematoxylin stain for formaldehyde-fixed tissue. *Arch. Pathol.* 32:446
4. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, str. 14-167.

Warnings and precautions regarding the materials contained in the product:
Not a hazardous substance or mixture acc. to Regulation (EZ) br. 1272/2008.

HPTA-IFU_ENV4, 24.02.2026., IŠP

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

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Version	Description / reason for change	Date
4	Revised acc. to Regulation (EU) 2017/746 - IVDR	24.02.2026.