P.A.S. DIASTASE KIT

IVD *In vitro* diagnostic medical device CE

Kit for detecting glycogen and mucopolysaccharide structures
INSTRUCTIONS FOR USE

REF Product code: PDIA-100T (for 100 tests)

Introduction

BioGnost's P.A.S. Diastase kit is most commonly used for identifying glycogen in liver. Periodic acid enables the molecules containing glycol groups to create aldehydes affected by Schiff's reagent staining them violet (magenta). Specific stains are created by applying the PAS method on unsubstituted polysaccharides, mucoproteins and glycoproteins, glycolipids and phospholipids. Alpha-amylase enzyme (also known as diastasis) is used for differentiation between glycogen and other PAS-positive structures by dissolving 1→4 glycosidic bonds, causing the glycogen to remain unstained after the PAS reaction. BioGnost's P.A.S. Diastase kit uses thermostable enzyme which does not require heating to +37°C to be active, but incubating the section at +37°C is preferred in order to achieve better glycogen breakdown. The same tissue section is used as negative control for this reaction, but the sample is not treated using alpha-amylase.

Product description

- **P.A.S. DIASTASE KIT** – Kit for differentiating glycogen from other PAS positive structures

The kit contains:	for 100 tests (PDIA-100T)	Storage temperature:
Alpha-amylase, solution	30 mL (ALF-OT-30)	2-8°C
Periodic acid, 0.8% solution	30 mL (PK08-OT-30)	15-25°C
BioSchiff reagent	30 mL (BS-OT-30)	15-25°C
Hematoxylin ML	30 mL (HEMML-OT-30)	15-25°C
Bluing reagent	30 mL (BR-OT-30)	15-25°C

Other sections and reagents necessary for testing:

- 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 BioClear New, an aliphatic hydrocarbon based xylene substitute
- Infiltration and embedding 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 types of BioGnost's VitroGnost glass slides

Preparing histological sections for staining

- Fix the tissue sample well (4% NB Formaldehyde, 10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (the Histanol range).
- Clear the sample with an intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and embed 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.

Sample staining procedure

NOTE

Apply the reagent so it completely covers the section.

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.	Apply the Alpha-amylase solution on the the section (add ≥5 drops)	15 - 20 minutes at room temperature (better glycogen breakdown achieved by heating at +37°C)
	Skip this step for negative control	
6.	Rinse the section under indirect stream of distilled (demi) water	3 min
7.	Apply Periodic acid, 0.8% solution (add ≥5 drops)	5-10 minutes
8.	Rinse the section under indirect stream of tap water	3 min
9.	Rinse the section in distilled (demi) water	
10.	Apply BioSchiff reagent (add ≥5 drops)	10-15 minutes
11.	Rinse under indirect stream of tap water	3 min
12.	Apply Hematoxylin ML (add ≥5 drops)	1-3 minutes
13.	Rinse under indirect stream of distilled water	10 seconds
14.	Nuclear bluing with Bluing reagent	
	Note: End the process of bluing after the nuclei turn blue	

15.	Immerse the sections in distilled/demineralized water.	
16.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
17.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
18.	Dehydrate using 100% alcohol (Histanol 100)	2 min
19.	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 covering/mounting over 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

Blue - nuclei
Red to purple - basal membrane, fungal cell wall
Magenta - glycogen (on negative control section), polysaccharides, neutral mucopolysaccharides, mucoproteins and glycoproteins, glycolipids, phospholipids, collagen
Lack of magenta (purple background) - site of glycogen breakdown

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

P.A.S Diastase kit's reagents have different storage temperature regime marked on their labels. .Keep at declared temperatures and in a dry place, do not freeze and avoid exposing to direct sunlight. In order to ensure the quality and shelf life of the BioSchiff reagent, keep it at 2-8°C after first opening Manufacturing and expiration date are printed on the product's label.

References

1. Culling, C.F.A.(1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Davey, F.R. et Nelson, D.A.(1977): Periodic Acid Schiff (PAS) Stain. IN Hematology, 2nd ed., W. J. Williams, E. Buetler, A. J. Erslev, R.W. Rundles, McGraw-Hill, New York, p 1630-1632.
3. Hotchkiss, R.D.(1948): A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations, *Arch. Biochem.* 16, p 131.
4. Sheehan D.C. et Hrapchak, B.B.(1980): Theory an Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

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Refer to the supplied documentation	Storage temperature range	Valid until	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
Refer to supplied instructions	Keep away from heat and sunlight	REF Product code	Manufacturer		
IVD For *in vitro* diagnostic use only	Keep in dry place	LOT Lot number			