BIOGNOST®

P.A.S. KIT

IVD In vitro diagnostic medical device CE

Five-reagent Periodic Acid-Schiff's kit

INSTRUCTIONS FOR USE

REF Catalog number: PAS5-100T (5 x 30 mL) PAS5-K-100 (5 x 100 mL) PAS5-K-500 (5 x 500 mL)

Introduction

One of the most frequently used chemical methods in histology is P.A.S. staining. The PAS staining is based on oxydation reaction with the presence of periodic acid and Schiff's reagent. Periodic acid makes the molecules containing glycol groups create aldehydes affected by Schiff's reagent that stains them violet (magenta). This method is most commonly used in liver and muscle cells testing. Specific stains are created by applying P.A.S. method on unsubstituted polysaccharides, mucoproteins and glycoproteins, glycolipids and phospholipids. Combined with Alcian Blue, it can detect acid mucosubstances (glycosaminoglycans).

Product description

- **P.A.S. KIT** – Aldehydes, mucopolysaccharides and mucoproteins staining kit

The kit contains:	100 tests (PAS5-100T)	5 x 100 mL (PAS5-K-100)	5 x 500 mL (PAS5-K-500)
Periodic acid, 0.8% solution	30 mL (PK08-OT-30)	100 mL (PK08-OT-100)	500 mL (PK08-OT-500)
BioSchiff reagent	30 mL (BS-OT-30)	100 mL (BS-OT-100)	500 mL (BS-OT-500)
Sodium metabisulphite, solution	30 mL (NM-OT-30)	100 mL (NM-OT-100)	500 mL (NM-OT-500)
HCL reagent, P.A.S.	30 mL (HCLP-OT-30)	100 mL (HCLP-OT-100)	500 mL (HCLP-OT-500)
Hematoxylin ML	30 mL (HEMML-OT-30)	100 mL (HEMML-OT-100)	500 mL (HEMML-OT-500)

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, 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
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparation of additional solutions used in staining

- **Sulfite solution**
  For use with kits for 100 tests: mix 0.25 ml of Sodium metabisulfite, solution with 0.25 ml of HCl reagent, PAS Add another 5 ml of tap water, then mix.
  For use with larger volume kits: mix 5 ml of Sodium metabisulfite, solution with 5 ml of HCl reagent, PAS Add 100 ml of tap water, then stir.
  Note: Prepare the sulfite solution shortly before using. Adjust the volume of prepared sulfite solution to the number of sections and the method of treating the sulfite solution in order to achieve more economic consumption and maximum efficiency.

Preparing the histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), 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 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.

NOTE

Apply the reagent so it completely covers the section.
The bottle containing BioSchiff reagent must be tightly closed in order to avoid SO_2 evaporation and to maintain the quality of the reagent.

Sample staining procedure

a) using kit for 100 tests (PAS5-100T)

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.	Treat with Periodic acid, 0.8% solution (add ≥5 drops)	5-10 minutes
6.	Rinse under tap water	3 min
7.	Rinse the section with distilled (demi) water	
8.	Treat with BioSchiff reagent (add ≥5 drops)	10-15 minutes
9.	Pour the reagent off the section without rinsing	
10.	Treat with sulfite solution (add ≥5 drops)	3 exchanges, 2 min each
	Note: apply sulfite solution to the section, then pour off the reagent from the section after 2 minutes, and repeat the procedure twice; do not rinse between exchanges	
11.	Rinse under tap water	3 min

12.	Stain using Hematoxylin ML (add ≥5 drops)	1-3 minutes
13.	Rinse under tap water	3 min
14.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
15.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
16.	Dehydrate using 100% alcohol (Histanol 100)	2 min
16.	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.

b) using five-reagent 100 mL or 500 ml kit (PAS5-K-100, PAS5-K-500)

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 Periodic acid, 0.8% solution.	5-10 minutes
6.	Rinse under tap water	3 min
7.	Rinse the section with distilled (demi) water	
8.	Immerse in BioSchiff reagent	10-15 minutes
	Note: during staining procedure it is required to put a lid on the jar in order to avoid SO_2 evaporation	
9.	Pour the reagent off the section without rinsing	
10.	Immerse into sulfite solution	3 exchanges, 2 min each
	Note: immerse the sections in 3 exchanges of sulfite solution; do not rinse between exchanges	
11.	Rinse under tap water	3 min
12.	Immerse into Hematoxylin ML	1-3 minutes
13.	Rinse under tap water	3 min
14.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
15.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
16.	Dehydrate using 100% alcohol (Histanol 100)	2 min
	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.

Result

Blue - nuclei
Violet - polysaccharides, glycogen, neutral mucopolysaccharides, mucoproteins, glycoproteins, glycolipids, phospholipids, basement membrane, collagen

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

Store the P.A.S. kit in a tightly closed original packaging at temperature stated on the product label. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label. Valid BioSchiff reagent solution is colorless. Discard after it starts to assume color because of the SO_2 loss.

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.

PAS5-X, V22-EN10, 27 April 2018, AK/IŠP

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