BIOGNOST®

P.A.S.M. / JONES KIT, STABILIZED

NEW STAINING PROCEDURE

IVD *In vitro* diagnostic medical device CE

Periodic-Acid-Silver-Methenamine kit for staining argentaffin structures and kidney membranes

INSTRUCTIONS FOR USE

REF Product code: PASM-100T (for 100 tests) PASM-K-100 (for 300-350 tests)

Introduction

P.A.S.M. / Jones kit is used in histology for visualizing argentaffin structures, especially kidney membranes, but also fungi and certain pathogen organisms. Staining procedure starts with periodic acid solution being used to oxidize 1,2-glycols to aldehydes. During incubation in silver-methenamine-borate working solution aldehydes are reduced and at the same time cause reduction of silver ions to metallic silver that manifests as brown to black structures on the section. This is followed by toning the solution with gold chloride solution that additionally improves staining intensity of target structures (fungi, basal membranes and others), and it reduces background staining. Excessive unbound silver-gold bonds is removed by rinsing the section with sodium thiosulfate solution. Finally, the sections are exposed to Nuclear Fast Red (Kernechtrot) counterstain that stains background structures red; that in turn creates clear and visually rich contrast to target structures (colored in brown-black).

This is new and improved formulation for P.A.S.M./Jones staining. Kit is stable at room temperature, do not store at lower temperature!

Product description

- **P.A.S.M. / JONES KIT -** Seven-reagent kit for staining argentaffin structures and kidney membranes.

The kit contains:	100 tests (PASM-100T)	300-350 tests (PASM-K-100)
Periodic acid, 1% solution	30 mL (PK1-OT-30)	100 mL (PK1-OT-100)
Silver nitrate, stabilized solution	3 x 100 mL (SNS-OT-100)	2 x 500 mL (SNS-OT-500)
Methenamine, solution	50 mL (MET-OT-50)	2 x 100 mL (MET-OT-100)
Borax, solution	35 mL (BO-OT-35)	105 mL (BO-OT-105)
Gold chloride, 0.6% solution	30 mL (ZK06-OT-30)	100 mL (ZK06-OT-100)
Sodium thiosulfate, 2% solution	30 mL (NT2-OT-30)	100 mL (NT2-OT-100)
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	100 mL (KR-OT-100)

CAUTION:

Adhere to the following rules in order to achieve the best results:
- use distilled or demineralized high purity water **WITHOUT** any chlorine
- use completely clean laboratory glassware
- do not touch the sections / be in contact with metal objects (scissors, tweezers etc.) during staining
- all the reagents must reach room temperature before use
- apply the reagents so they completely cover the section

Preparation of silver-methenamine-borate working solution:

a) 40 ml volume (optimal for Coplin jar):

Add 15 ml of double-distilled (demi) water, then add 3 ml of Methenamine, solution and 2 mL of Borax, solution. Then add 20 mL of Silver nitrate, solution and stir by using glass stick.

b) 80 ml volume (optimal for Hellendahl jar):

Add 30 ml of double-distilled (demi) water, then add 6 ml of Methenamine, solution and 4 mL of Borax, solution. Then add 40 mL of Silver nitrate, solution and stir by using glass stick.

NOTE: use silver-methenamine-borate working solution for single staining only and discard after use

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 Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 1-3 μm sections and place them on the VitroGnost glass slide. We recommend adhesive glass slides (VitroGnost Plus Ultra, VitroGnost PLL, VitroGnost SIL, VitroGnost Super Frost Plus)

Sample staining procedure – NEW 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, 3 and 2 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Add Periodic acid, 1% solution (≥5 drops)	11 min
	Note: shorten incubation period to achieve fungi oxidation	5 min
6.	Rinse in double distilled (demi) water	3 exchanges, 30 seconds each

7.	Prepare **methenamine-silver-borate working solution** and incubate with the sections at +56°C in a water bath. Check the section staining microscopically if necessary.	30 - 35 min
	Note: for staining fungi, incubate for 20 min and visually check until required staining intensity is achieved (fungi turn dark brown on light yellow background)	20 - 25 min for staining fungi
8.	Rinse in redistilled (demi) water (room temperature)	3 exchanges, 30 seconds each
9.	Add Gold chloride, 0.6% solution (≥5 drops)	30 - 60 seconds
	Note: longer exposure period to Gold chloride, 0.6% solution shifts the membrane staining hue from black to gray	
10.	Rinse in redistilled (demi) water (room temperature)	3 exchanges, 30 seconds each
11.	Add Sodium thiosulfate, 2% solution (≥5 drops)	2 min
12.	Rinse well under tap water	2 min
13.	Add Nuclear Fast Red (Kernechtrot) reagent (>5 drops)	5-10 minutes
	Note: depending on personal preferences, instead of Nuclear Fast Red (Kernechtrot) reagent for counterstaining it is possible to stain by using Hematoxylin and Eosin solutions already routinely used in the laboratory	
14.	Rinse in distilled (demi) water	
15.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
16.	Dehydrate using 100% alcohol (Histanol 100)	30 seconds
17.	Dehydrate using 100% alcohol (Histanol 100)	2 min
18.	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

Basal membranes, glycogen, bacteria and fungi - brown to black
Background - pink

Note

Histology staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

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

Keep P.A.S.M kit in a tightly closed original package at temperatures between +15°C and +25°C. Keep in a dry place, do not freeze and avoid exposure to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Bancroft, J.D., Gamble, M. Livingstone, C. Theory and practice of Histological Techniques – 5° edizione 2002.
2. Grocott. A Stain for fungi in tissue section and smears. Am J Pathol. 1955; 25:975.
3. Koski, J.P. (1981): Silver methenamine-borate (SMB): Cost reduction with technical improvement in silver nitrate-gold chloride impregnations. J. Histotechnol. 4; p 115.
4. Melis, M., Carpino, F., Di Tondo, U., Ermes, E. Techniche in anatomia patologica. 1989.

PASM-X, V11-EN12, 23 November, 2021., KB/IŠP

	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
	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						