BIOGNOST®

BIOSCHIFF REAGENT

IVD *In vitro* diagnostic medical device CE

Schiff's reagent for detection of aldehydes and mucous substances
For use in microscopy and electrophoresis

INSTRUCTIONS FOR USE

REF Catalog number: BS-OT-30 (30 ml) BS-OT-100 (100 ml) BS-OT-500 (500 ml) BS-OT-1L (1000 mL)

Introduction

BioSchiff reagent is a colorless solution that changes to violet (magenta) in the presence of aldehydes. The intensity of the color obtained depends on the amount of reactive glycol structures in the tissue. It is prepared by the reduction of pararosaniline by using sulfuric acid. Schiff's reagent is used with various chemical methods, and one of the most common and most widely used ones is P.A.S. staining (Periodic Acid Schiff). The P.A.S. staining is based on oxidation 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. Schiff's reagent can be used for DNA detecting according to Feulgen.

Product description

- **BIOSCHIFF REAGENT** - Pararosaniline, hydrochloric acid and sodium metabisulfite solution with added stabilizer.

Other slides and reagents that may be used in staining:

- Fixative such as BioGnost's neutral buffered formalin: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Histopathology staining reagent, such as BioGnost's Hematoxylin M, Hematoxylin G2, and Hematoxylin G3
- Oxidation reagent, such as BioGnost's Periodic acid, 0.5% solution
- Reagents for creating sulfite solution, such as BioGnost's Sodium metabisulfite, solution and HCL reagent, P.A.S.
- Clearing agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- 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
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm

Preparation of additional solutions used in staining

- Sulfite solution
  Mix 10 ml of Sodium metabisulfite, solution with 10 ml of HCL reagent, P.A.S. Add another 200 ml of tap water, then mix.
  Note: Prepare the sulfite solution shortly before using.

Preparing 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 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.

P.A.S. 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.	Treat with Periodic acid, 0.5% solution (add ≥5 drops)	5 min
6.	Rinse under tap water	3 min
7.	Rinse the section with distilled (demi) water	
8.	Treat with BioSchiff reagent (add ≥5 drops)	15 min
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 M (add ≥5 drops)	2 min
	Note: it is possible to use Hematoxylins G2 and G3 instead Hematoxylin M for staining cellular nuclei as contrast to PAS-positive structures	
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
17.	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, 0.5% solution.	5 min
6.	Rinse under tap water	3 min
7.	Rinse the section with distilled (demi) water	
8.	Immerse in BioSchiff reagent	15 min
	Note: during staining procedure it is required to put a lid on the jar in order to avoid evaporation$SO_{2\ evaporation}$	
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 in Hematoxylin M	2 min
	Note: it is possible to use Hematoxylins G2 and G3 instead Hematoxylin M for staining cellular nuclei as contrast to PAS-positive structures	
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

Keep BioSchiff reagent in a tightly closed original package at room temperature. In order to ensure the quality and shelf life of the reagent, keep it at 2-8°C after first opening. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Bancroft, J. D. et Gamble, M. (2002): Theory and Practice of Histological Techniques, 5th ed., Churchill Livingstone, London.
2. Kiernan, J.A. (1999): Histological and histochemical methods: Theory and practice, 3rd ed., Butterworth Heinemann, Oxford, UK.
3. Kodousek, R. (1969): A new, rapid method of preparing Schiff's reagent, *Histochemical Journal,* 1, p 277-278.

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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	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						