BIOGNOST®

WARTHIN STARRY KIT

IVD *In vitro* diagnostic medical device CE

Five-reagent kit for staining *Helicobacter pylori*

INSTRUCTIONS FOR USE

REF Catalogue number: WS-12T (for staining **12-48** sections)

Introduction

BioGnost's Warthin Starry kit enables staining histology sections and *Helicobacter pyilori* visualization in a simple way and in a few steps. The kit is distinctive because it contains 12 containers with gelatin. They can also be used for practical incubation and section staining. By adding other reagents it is possible to create active developing solution used for immersing sections and simultaneous staining of 1 to 4 sections. Staining using Warthin Starry kit is based on reducing silver nitrate to silver using hydroquinone. The formed silver is deposited on the surface of *Helicobacter pylori*. The microscopic image shows the bacteria stained dark brown to black, the cells are stained yellow-brown, and the nuclei brown. The bacteria may be detected in mucus of surface epithelium, in apical glands of the stomach, and in mucosa of the stomach.

Product description

•**WARTHIN STARRY KIT** – Kit for staining *Helicobacter pylori* in histologic paraffin sections

The kit contains:	12 tests for 12-48 sections (WS-12T)	Storage temperature
Acid solution	500 mL (KOT-OT-500)	15-25°C
Silver nitrate, crystals	5 g (SNP-P-5)	15-25°C
Silver nitrate, WS solution	3 mL (SNWS-OT-3)	2-8°C
Gelatin, solution (in transport / incubation container)	12 x 8 mL (GEL-OT-8)	2-8°C
Hydroquinone, solution	5 mL (HQ-OT-5)	2-8°C

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

Preparing 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.

Histological sections 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.	Preparation of **impregnation solution:** add Silver nitrate, crystals into the bottle with Acid solution, and dissolve them by rotating the bottle.	
	Note: prepared impregnation solution is stable for at least 1 year if stored at temperature of 2-8°C. Let the solution reach room temperature before each use (only the volume to be used for staining). In time small vlack spots may appear at the bottom of the solution; they do not influence staining. We do not recommend filtrating the solution because impurities from filter paper may contaminate the solution.	
6.	Pour 40 ml of impregnating solution into the staining jar (we recommend Coplin staining jars) Note: do not reuse the impregnating solution	
7.	Impregnate 1 to 4 sections by immersing them into the impregnating solution. Incubate in a dark room at 50 °C	40 min
8.	Cool the sections down to room temperature	
9.	**Very important:** Prepare the **developing solution** during the last 12 minutes of incubation in the impregnating solution. Heat the container with gelatin in transport / incubation container at 50 °C for 10 min. Add 4 drops of Silver nitrate, WS solution into the container with dissolved gelatin. Mix shortly by rotating the container. Add 7 drops of Hydroquinone, solution and stir again.	
10.	Stain the sections by immersing them into the container with developing solution and incubate at 50 °C in an upright position. Macroscopically check the sections and stop the incubation after the sections are stained yellow-brown	5-10 minutes
11.	Rinse well in warm running water	2 min
12.	Dehydrate using 70% alcohol (Histanol 70)	2 exchanges, 30 seconds each
13.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
14.	Dehydrate using 100% alcohol (Histanol 100)	30 seconds
15.	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 a VitroGnost cover glass.

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.
Section staining may also be conducted by using the microwave oven (incubation period is up to 4 times shorter). We suggest standardizing the method due to various power outputs of microwave ovens.

Result

Black – *Helicobacter pylori*
Brown-red - background

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

Components of Warthin Starry kit are kept under different storage conditions. Keep reagents dry, at temperature indicated on the label in a tightly sealed original packaging. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry 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. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

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	Refer to the supplied documentation	°C	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						