BIOGNOST®

ALCIAN YELLOW TOLUIDINE BLUE KIT

IVD In vitro diagnostic medical device

Six-reagent kit for staining Helicobacter pylori in stomach samples

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INSTRUCTIONS FOR USE

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

Introduction

Alcian Yellow Toluidine Blue is used for visualization of *H. pylori* found in endoscopic and surgical stomach samples. In first step Alcian Yellow is specifically bound for previously oxidized mucins; gastric bacteria are stained afterward, as well as cellular nuclei and other structures using Toluidine Blue. This provides excellent contrast between dark blue stained bacteria placed on yellow surface and extracellular foveolar mucins.

Product description

Alcian Yellow Toluidine Blue kit - Six-reagent kit for staining H. pylori found in histology stomach sections

The kit contains:	100 tests (AYTB-100T)
Periodic acid, 1% solution	30 mL (PK1-0T-30)
HCL reagent, A.T.	30 mL (HCLAT-0T-30)
Sodium metabisulphite, solution	30 mL (NM-OT-30)
Alcian Yellow, solution	30 mL (AY-0T-30)
Buffer solution, A.T.	30 mL (PO-OT-30)
Toluidine Blue reagent	30 mL (TBR-0T-30)

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 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

Preparing the histological sections for staining

- Fixate 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, BioWax 56/58, BioWax Blue, BioWax Micro)
- Cut the paraffin block to 4-6 μ m slices and place them on a VitroGnost glass slide

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

1	Deparaffinize the section in vylene (BioClear) or in a vylene substitute (BioClear New)	3 exchanges, 5 min				
		each				
2.	Pahydrata ucing 100% alaabal (Historial 100)	2 exchanges, 5 min				
		each				
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min				
4.	Rehydrate in distilled (demi) water	2 min				
5.	Add 6 drops of Periodic acid, 1% solution	let it set for 10 min				
6.	Rinse with distilled water thoroughly					
7.	Add 3 drops of HCI reagent, A. T. and 3 drops of Sodium metabisulfite, solution, then gently mix the solutions on	let it set for 5 min				
	the section					
8.	Rinse with distilled water thoroughly					
9.	Add 6 drops of Alcian Yellow, solution	let it set for 5 min				
10.	Rinse in distilled water thoroughly					
44	Add 6 drops of Buffer solution, A.T. and 2 drops of Toluidine Blue reagent, gently mix the solutions on the	lat it cat for 3 min				
	section					
12.	Rinse with distilled water thoroughly					
13.	Let the sections dry on air until the tissue changes color from purple to blue	20-30 min				
14	Clear the section in vulene (BioClear) or in a vulene substitute (BioClear New)	3 exchanges, 2 min				
14.	טובמו נוום שבטמטו ווו אאובווים (שוטטובמו) טו ווו מ אאובווים שששטונענים (שוטטובמו ועביא)	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

Bacteria - blue-black Mucins - yellow to green Nuclei and other cellular structures - hues of blue

Note

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 Yellow Toluidine Blue kit in a tightly sealed original packaging at temperature of 15°C to 25 °C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

- 1. Prophet, E.B., Mills, B., Arrington, J., Sobin, L. (1968) Laboratory methods in histotechnology. McGraw Hill, Washington D.C.
- 2. Bancroft, J.D., Gamble, M. (2002) Theory and practice of Histological Techniques. Churchill Livingstone, New York.
- 3. Kiernan, J.A. (2015) Histological and Histochemical Methods, Theory and Practice, 5th ed. Bunbury, Oxford, UK.

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Â	Refer to the supplied documentation	°C 🔓	Storage temperature range	\sum	Number of tests in package	REF	Product code	CE	European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb	(E
Ē	Refer to supplied instructions	×	Keep away from heat and sunlight		Valid until	LOT	Lot number	** *	Manufacturer		CROATIA www.biognost.com		
IVD	For <i>in vitro</i> diagnostic use only	-	Keep in dry place	ų	Caution - fragile					-			

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