BIOGNOST®

ELASTICA-VAN GIESON KIT

IVD *In vitro* diagnostic medical device CE

Four-reagent kit for rapid staining of collagen fibers

INSTRUCTIONS FOR USE

REF Catalogue number: WVGB-100T (100 tests) WVGB-K-100 (4 x 100 mL)

Introduction

Elastica-Van Gieson kit is used for staining elastin, connective tissue and collagen. Elastic fibers consist of elastin polymers and elastic microfibrils that make up a 3D network in an extracellular matrix inside connective tissue (skin, elastic cartilage, vascular walls, lung tissue and in vocal cords). Unlike standard histology stains, Weigert Van Gieson reagent (known as resorcin-fuchsin reagent) displays selective differentiation of tissue samples, even in early phase of disease. When using Elastica-Van Gieson kit, the sections are first treated with Resorcine Fuchsin reagent. Positively charged hydrophobic resorcin-fuchsin dye is present in large amounts and it is deposited owing to electropolarity to acid, negatively charged shells of elastic fibers. After differentiation in diluted alcohol or rinsing in tap water, the nuclei are stained with acid-fast Weigert hematoxylin. The last phase of staining is staining with Fuchsin Acid Van Gieson reagent that contains two dyes (acid fuchsin, picric acid) that simultaneously stain different tissue structures. Acid fuchsin stains collagen fibers intensive red while picric acid stains muscle fibers, erythrocytes and glial fibers yellow. Sections are optimally stained in short period of time by using rapid staining method.

Product description

- **ELASTICA-VAN GIESON KIT** – Kit for rapid staining of elastic fibers.

The kit contains:	100 tests (WVGB-100T)	4 x 100 mL (WVGB-K-100)
Resorcine Fuchsin reagent	60 mL (RFR-OT-60)	100 mL (RFR-OT-100)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)
Ferri reagent, Weigert B	30 ml (FR-OT-30)	100 ml (FR-OT-100)
Fuchsin Acid Van Gieson reagent	30 mL (FAG-OT-30)	100 mL (FAG-OT-100)

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 Plus56/58, 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

NOTE

Apply the reagent so it completely covers the section.
In order to avoid reagent evaporation from the section, we recommend using incubation chamber/plate.

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.

a) using kit for 100 tests (WVGB-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.	Staining with Resorcine Fuchsin reagent: dip the section in Resorcine Fuchsin reagent and cover it to prevent reagent evaporation. Reagent may be filtered and reused.	30 min
6.	Rinse under tap water	1 min
7.	Apply 5 drops of Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react.	5 min
8.	Rinse under tap water	1 min
9.	Drip Fuchsin Acid Van Gieson reagent (≥5 drops)	5-10 minutes
10.	Rinse quickly in distilled (demi) water	
11.	Quickly dehydrate through 96% and 100% alcohol (Histanol 96 and Histanol 100)	
	Note: the amount of yellow dye rinsed rises the longer the sections stays immersed	
12.	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.

a) using kit with six 100 ml reagents (WVGB-K-100)

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.	Staining with Resorcine Fuchsin reagent: dip the section in Resorcine Fuchsin reagent and cover it to prevent reagent evaporation. Reagent may be filtered and reused.	30 min
6.	Rinse under tap water	1 min
7.	Prepare Weigert hematoxylin working solution: mix equal volumes of Hematoxylin, Weigert A and Ferri reagent, Weigert B Note: working solution is stable for approximately 2 weeks. Prepare the working solution of volume adequate for staining test sections	
8.	Immerse into Weigert hematoxylin working solution and let it react	5 min
9.	Rinse under tap water	1 min
10.	Immerse into Fuchsin Acid Van Gieson reagent	5-10 minutes
11.	Rinse quickly in distilled (demi) water	
12.	Quickly dehydrate through 96% and 100% alcohol (Histanol 96 and Histanol 100)	
	Note: the amount of yellow dye rinsed rises the longer the sections stays immersed	
13.	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.

Results

Black-blue - nuclei
Hues of red-pink - collagen
Dark purple-black - elastic fibers
Yellow - connective tissue, erythrocytes and muscle tissues

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 Elastica-Van Gieson kit in a tightly closed original package at temperature between +15°C and +25°C. 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. Lillie, R.D. (1945): Studies on selective staining of collagen with acid aniline dyes, J. Technical Methods, 25:1
3. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.
4. Van Gieson, I. (1889): Laboratory notes of technical methods for the nervous system, New York Med. J., 50: 57-60

WVGB-X, V1-EN2, 02 July 2019, AK/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
i	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						