

WEIGERT-VAN GIESON KIT

IVD In vitro diagnostic medical device

Six-reagent kit for staining elastic fibers with prolonged incubation period INSTRUCTIONS FOR USE

REF Catalogue number: WVG-100T (for 100 tests)

Introduction

Weigert-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 dye) displays selective differentiation of tissue samples, even in early phase of disease. Prolonged incubation period causes excellent elastic fibers imaging selectivity.

WVG-K-100 (6 x 100 mL)

Product description

• WEIGERT-VAN GIESON KIT - Kit for staining elastic fibers.

The kit contains:	100 tests (WVG-100T)	6 x 100 mL (WVG-K-100)
Periodic acid, 0.8% solution	30 mL (PK08-0T-30)	100 mL (PK08-0T-100)
Resorcine Fuchsin reagent	60 mL (RFR-0T-60)	100 mL (RFR-0T-100)
HCL reagent, Weigert-Van Gieson	30 mL (HCLW-0T-30)	100 mL (HCLW-0T-100)
Hematoxylin, Weigert A	30 ml (HEMA-0T-30)	100 ml (HEMA-OT-100)
Ferri reagent, Weigert B	30 ml (FR-0T-30)	100 ml (FR-0T-100)
Fuchsin Acid Van Gieson reagent	30 mL (FAG-OT-30)	100 mL (FAG-0T-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 Plus 56/58, 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, BioMount DPX, BioMount DPX, BioMount DPX Low, BioMount DPX Low, BioMount DPX Low, BioMount DPX Low, BioMount C, BioMount Aqua
- 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.

Histological sections staining procedure

a) using kit for 100 tests (WVG-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.	Drip Periodic acid, 0.8% solution (\geq 5 drops)	5 min
6.	Staining with Resorcine Fuchsin reagent: dip the section in Resorcine Fuchsin reagent and cover it to prevent reagent evaporation. Leave at room temperature overnight or 60 min at $+45^{\circ}$ C in incubation tray. Reagent may be filtered	
	and reused.	
7.	Drip HCL reagent, Weigert-Van Gieson (≥5 drops)	10 min
8.	Rinse in distilled (demi) water	
9.	Apply 5 drops of Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react.	8-10 minutes
10.	Rinse under tap water	10 min
11.	Drip Fuchsin Acid Van Gieson reagent (≥5 drops)	30 sec
12.	Dehydrate through 95% alcohol (Histanol 95)	2 exchanges, 5 dips each
13.	Dehydrate through 100% alcohol (Histanol 100)	2 exchanges, 2 min each
14.	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 (WVG-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.	Immerse into Periodic acid, 0.8% solution.	5 min
6.	Staining with Resorcine Fuchsin reagent: dip the section in Resorcine Fuchsin reagent and cover it to prevent reagent evaporation. Leave at room temperature overnight or 60 min at $+45$ °C in incubation tray. Reagent may be filtered and reused.	
7.	Immerse into HCL reagent, Weigert-Van Gieson	10 min
8.	Rinse in distilled (demi) water	
9.	Prepare the 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	
10.	Immerse into Weigert hematoxylin working solution and let it react	8-10 minutes
11.	Rinse under tap water	10 min
12.	Immerse into Fuchsin Acid Van Gieson reagent (≥5 drops)	30 sec
13.	Dehydrate through 95% alcohol (Histanol 95)	2 exchanges, 5 dips each
14.	Dehydrate through 100% alcohol (Histanol 100)	2 exchanges, 2 min each
15.	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-brown - nuclei Hues of red pink - collagen Dark blue-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

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Keep Weigert-Van Gieson kit in a tightly closed original package at temperature between $+15^{\circ}$ C and $+25^{\circ}$ 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

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	(Ξ
(iii)	Refer to supplied instructions	*	Keep away from heat and sunlight	\square	Valid until	LOT	Lot number	444	Manufacturer		CROATIA www.biognost.com		
IVD	For <i>in vitro</i> diagnostic use only	-	Keep in dry place	4	Caution - fragile					-			