BIOGNOST®

VERHOEFF KIT

IVD *In vitro* diagnostic medical device CE

Six-reagent kit for staining elastic fibers

INSTRUCTIONS FOR USE

REF Catalogue number: VER-100T (for 100 tests) VER-K-100 (6x100 mL)

Introduction

Verhoeff kit is used primarily for staining elastin. However, it may be used for staining muscle fibers, as well as 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). It can be used instead of Weigert-Van Gieson kit. Visualization of elastic fibers is especially important in cases of emphizema (elastic tissue atrophy), arteriosclerosis (thinning and loss of elastic fibers), and many other cardiovascular diseases.

Product description

- **VERHOEFF KIT** – Kit for staining elastic fibers.

The kit contains:	100 tests (VER-100T)	6 x 100 mL (VER-K-100)
Hematoxylin, Verhoeff A	30 mL (HEMV-OT-30)	100 mL (HEMV-OT-100)
Ferri reagent, Verhoeff B	30 mL (FRV-OT-30)	100 mL (FRV-OT-100)
Iodine solution, Verhoeff C	30 mL (JODV-OT-30)	100 mL (JODV-OT-100)
Reagent for differentiation, Verhoeff	30 mL (RDV-OT-30)	100 mL (RDV-OT-100)
Sodium thiosulfate, 5% solution	30 mL (NT5-OT-30)	100 mL (NT5-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 Plus, 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.

Histological sections staining procedure

a) using kit for 100 tests (VER-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.	Add 7 drops of Hematoxylin, Verhoeff A, 3 drops of Ferri reagent, Verhoeff B, and 3 drops of Iodine solution, Verhoeff C. Place the section in the incubation tray in order to prevent evaporation.	30-60 minutes
	Note: prolonged exposition period (up to 60 minutes) intensifies staining	
6.	Rinse in distilled (demi) water	
7.	Add Reagent for differentiation, Verhoeff (≥5 drops) and differentiate the section	1-2 minutes
	Note: quickly rinse the section in distilled (demi) water after differentiation and microscopically check for the section for elastin being stained black. Repeat the differentiation if necessary	
8.	Rinse in distilled (demi) water	
9.	Add Sodium thiosulfate, 5% solution (≥5 drops)	1 min
10.	Rinse in distilled (demi) water	
11.	Drip Fuchsin Acid Van Gieson reagent (≥5 drops)	1-5 minutes
	Note: Fuchsin Acid Van Giesion is a counterstain; prolonged exposition period (up to 5 minutes) provides more intensive background staining	
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.

a) using kit with six 100 ml reagents (VER-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.	Add 20 mL of Hematoxylin, Verhoeff A, 8 mL of Ferri reagent, Verhoeff B, and 8 mL of Iodine solution, Verhoeff C. Place the section in the incubation tray and cover it in order to prevent evaporation.	30-60 minutes
	Note: prolonged exposition period (up to 60 minutes) intensifies staining. Discard of the prepared solution after use	
6.	Rinse in distilled (demi) water	
7.	Immerse in Reagent for differentiation, Verhoeff and differentiate the section	1-2 minutes
	Note: quickly rinse the section in distilled (demi) water after differentiation and microscopically check for the section for elastin being stained black. Repeat the differentiation if necessary	
8.	Rinse in distilled (demi) water	
9.	Immerse into Sodium thiosulfate, 5% solution	1 min
10.	Rinse in distilled (demi) water	
11.	Immerse into Fuchsin Acid Van Gieson reagent	1-5 minutes
	Note: Fuchsin Acid Van Giesion is a counterstain; prolonged exposition period (up to 5 minutes) provides more intensive background staining	
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.

Result

Purple-black - elastic fibers
Black-brown - nuclei
Hues of red pink - collagen
Yellow - connective tissue

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 Verhoeff 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. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.
3. Van Gieson, I. (1889): Laboratory notes of technical methods for the nervous system, New York Med. J., 50: 57-60

VER-X, V2-EN4, 13 September 2019, AK/VR

	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						