

# VAN GIESON TRICHROME KIT

**IVD** *In vitro* diagnostic medical device

CE

## Three-reagent set for staining collagen fibers

### INSTRUCTIONS FOR USE

**REF** Catalogue number: VG-100T (for 100 tests)      VG-K-100 (3 x 100 mL)      VG-K-500 (3 x 500 mL)

#### Introduction

Van Gieson trichrome kit is used for staining collagen, muscle tissue, keratinized epithelium, cytoplasm, glial fibers, and erythrocytes. Fuchsin Acid Van Gieson is a component of the kit and it 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. Amyloids, hyalin, colloid and mucosa are stained in nuances between red and yellow. Hematoxylin, Weigert A and Ferri reagent make up Weigert hematoxylin that creates stable cellular nuclei coloration.

#### Product description

- **VAN GIESON TRICHROME KIT** – Kit for staining collagen connective tissue

The kit contains:	100 tests (VG-100T)	3 x 100 mL (VG-K-100)	3 x 500 mL (VG-K-500)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)	500 mL (HEMA-OT-500)
Ferri reagent, Weigert B	30 ml (FR-OT-30)	100 ml (FR-OT-100)	500 mL (FR-OT-500)
Fuchsin Acid Van Gieson reagent	30 mL (FAG-OT-30)	100 mL (FAG-OT-100)	500 mL (FAG-OT-500)

#### 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: Histolanol 70, Histolanol 80, Histolanol 95 and Histolanol 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 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

#### Preparation of additional solutions used in staining

- Weigert hematoxylin working solution  
Mix Hematoxylin, Weigert A and Ferri reagent, Weigert B in 1:1 ratio. Working solution is stable for approximately 2 weeks. Prepare the working solution of volume adequate for staining test sections.

#### 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 (Histolanol 70, Histolanol 80, Histolanol 95 and Histolanol 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  $\mu$ m slices and place them on a VitroGnost glass slide.

#### Histological sections staining procedure

##### a) using kit for 100 tests (VG-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 (Histolanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histolanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Apply 5 drops of Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react.	5 min
6.	Rinse under tap water	3 min
7.	Staining with Fuchsin Acid Van Gieson reagent  Note: Fuchsin Acid Van Gieson is a counterstain; prolonged exposition period (up to 5 minutes) provides more intensive background staining	3-5 min
8.	Quickly dehydrate through 96% and 100% alcohol (Histolanol 96 and Histolanol 100)	
9.	Note: the amount of yellow dye rinsed rises the longer the sections stays immersed	
10.	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.

\* To avoid section fading (and consequently loss of yellow), we recommend clearing in xylene (BioClear) and mounting with BioMount DPX or BioMount DPX New media.

## b) using kit with six 100 ml reagents (VG-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 using Weigert hematoxylin working solution	30-60 minutes
	Rinse under tap water	
6.	Staining with Fuchsin Acid Van Gieson reagent	3-5 minutes
7.	Note: Fuchsin Acid Van Gieson is a counterstain; prolonged exposition period (up to 5 minutes) provides more intensive background staining	
	Quickly dehydrate through 96% and 100% alcohol (Histanol 96 and Histanol 100)	
8.	Note: the amount of yellow dye rinsed rises the longer the sections stays immersed	
9.	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.

\* To avoid section fading (and consequently loss of yellow), we recommend clearing in xylene (BioClear) and mounting with BioMount DPX or BioMount DPX New media.

### 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

Blue-brown - nuclei

Red - collagen

Yellow - muscle fiber, glial fibers

Hues between yellow and red - colloid, mucosa, hyalin, amyloid, keratinized epithelium

### 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 Van Gieson trichrome 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, 2<sup>nd</sup> 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, 2<sup>nd</sup> 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

VG-K-X, V8-ENG, July 11, 2022, KB/ŠP

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				

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