BIOGNOST®

PERLS-VAN GIESON KIT

IVD *In vitro* diagnostic medical device CE

Kit for detecting free iron ferric (Fe^{3+}) ions and connective tissue

INSTRUCTIONS FOR USE

REF Product code: PEG-100T (for 100 tests) PEG-K-100 (3 x 100 mL)

Introduction

Perls-Van Gieson kit is used for staining free iron ferric ions and connective tissue. 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. Potassium hexacyanoferrate solution reacts in presence of iron and creates non-soluble blue dye precipitate.

Product description

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

The kit contains:	100 tests (PEG-100T)	3 x100 ml (PEG-K-100)
Potassium hexacyanoferrate, solution	30 mL (KHC-OT-30)	100 mL (KHC-OT-100)
HCl reagent, HemoGnost Perls	30 mL (HCLH-OT-30)	100 mL (HCLH-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 C, BioMount Aqua or 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 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.

Working solution preparation (for staining procedure marked under b)

Immediately before staining mix Potassium hexacyanoferrate, solution and HCl reagent, HemoGnost Perls in 1:1 ratio.

NOTE

Apply the reagent so it completely covers the section.

Histological sections staining procedure:

a) using kit for 100 tests (PEG-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 ≥5 drops of Potassium hexacyanoferrate, solution and ≥5 drops of HCL reagent, HemoGnost Perls. Gently stir.	20 min
6.	Carefully rinse in distilled water	
7.	Stain using Fuchsin Acid Van Gieson reagent (add ≥5 drops)	3-5 minutes
	Note: Fuchsin Acid Van Giesion is a counterstain; prolonged exposition period (up to 5 minutes) provides more intensive background staining	
8.	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	
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 VitroGnost cover glass.

b) using three-reagent 100 mL kit (PEG-K-100)

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 in working solution (mixture of HCl reagent and Potassium hexacyanoferrate)	20 min
6.	Carefully rinse in distilled water	
7.	Immerse into Fuchsin Acid Van Gieson reagent	3-5 minutes
	Note: Fuchsin Acid Van Giesion is a counterstain; prolonged exposition period (up to 5 minutes) provides more intensive background staining	
8.	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	
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 VitroGnost cover glass.

Result

Ferric ions - blue
Collagen - purple red
Other tissues and cells - yellow

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.

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 Perls-Van Gieson Trichrome kit in a tightly sealed original packaging at temperature of +15 to +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

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	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
	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						