IRON CHLORIDE, SOLUTION

IVD *In vitro* diagnostic medical device CE

For staining with Fouchet-Van Gieson kit

INSTRUCTIONS FOR USE

REF Product code: FK-OT-100 (100 mL)

Introduction

Fouchet-Van Gieson kit is used for simultaneous visualization of bilirubin and collagen in histological samples. Bilirubin is a yellow-brown pigment created as a result of hemoglobin degradation. Hemoglobin degradation occurs in bone marrow, spleen, and liver. In case of patients that suffer from hepatitis, bilirubin builds up in the form of thrombus in bile ducts and in form of granules in hepatocytes and in cytoplasm of Küpfer cells. Pigment is insoluble in water and in water fixatives. However, in case of too long exposure to formalin fixatives it may turn green. The color that is created during staining using Fouchet-Van Gieson kit is due to strong oxidoreduction of the complex and subsequent conversion to biliverdin (green). False positive reaction may be checked using HemoGnost Perls kit - in that case Perls reaction will always be negative to bilirubin.

Product description

- **IRON CHLORIDE, SOLUTION** - Iron chloride hexahydrate aqueous solution

Example of using Iron chloride, solution as a Fouchet-Van Gieson kit component

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 56/68, BioWax Blue.
- 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
- BioGnost's reagents that comprise Fouchet-Van Gieson kit: Trichloroacetic acid, solution (TKO-OT-100) and Fuchsin Acid Van Gieson reagent (FAG-OT-100)

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), 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).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

Procedure of staining histology samples by using Fouchet-Van Gieson Trichrome kit with three 100 ml reagents (FVG-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.	Mix equal volumes of Trichloroacetic acid, solution and Iron chloride, solution, and immerse the sections	5 min
	Note: the solution can be used for further few months; however, the best results are achieved with freshly prepared solution	
6.	Rinse in distilled (demi) water	
7.	Immerse into Fuchsin Acid Van Gieson reagent	7 min
8.	No rinsing, dry on air	5 min
9.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
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 VitroGnost cover glass.

*To avoid section fading (loss of yellow) we recommend clearing in xylene (BioClear) and mounting a glass slide using BioMount DPX or BioMount DPX New medium.

Result

Bilirubin - green
Collagen - red
Muscle tissue, glial fibers, cytoplasm, corneal epithelium - 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 Iron chloride, solution in a tightly sealed original packaging at temperature of +2°C to +8°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, 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
	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				

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