BIOGNOST®

COLLOIDAL IRON KIT

IVD *In vitro* diagnostic medical device CE

Six-reagent kit for visualizing acid mucopolysaccharides and proteoglycans

INSTRUCTIONS FOR USE

REF Product code: KOL-100T (for 100 tests)

Introduction

Colloidal iron kit is used for visualization of carboxylated and sulphated groups of acid mucins and proteoglycans. This method is based on the principle of binding positively charged ferric ions (Fe^{3+}) to negatively charged endings of acid mucopolysaccharides and proteoglycans. Excessive reagents are rinsed while the bound ferric ions get visualized using Prussian Blue reaction that using potassium ferrocyanide causes light blue precipitations of iron ferrocyanide to appear. Finally, the sections are exposed to Van Gieson stain that selectively stains different tissue structures and in turn creates clear and visually rich contrast. The method can be combined with the P.A.S. method; that way glycogen and neutral mucopolysaccharides would get differentially stained characteristically magenta.

Product description

- **COLLOIDAL IRON KIT** – six-reagent kit for visualizing acid mucopolysaccharides and proteoglycans

The kit contains:	100 tests (KOL-100T)
Acetic acid, Colloidal Iron (A) solution	250 mL (OKA-OT-250)
Acetic acid, Colloidal Iron (B) solution	2 x 30 mL (OKB-OT-30)
Colloidal Iron, stock solution	30 mL (KOL-OT-30)
Potassium hexacyanoferrate, Colloidal Iron solution	30 mL (KHCK-OT-30)
HCL reagent, Colloidal Iron	30 mL (HCLK-OT-30)
Fuchsin Acid Van Gieson reagent	30 mL (FAG-OT-30)

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 52/54, BioWax Plus 56/58, 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, or 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
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

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 Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

Sample staining procedure

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.	Rinse in Acetic acid, Colloidal Iron (A) solution	30 seconds
6.	Add ≥15 drops of Acetic acid, Colloidal iron (B) solution, and ≥5 drops of Colloidal Iron, stock solution. Gently stir. Note: Use fresh solution, discard after use.	30 min
	Carefully rinse the working solution in Acetic acid, Colloidal Iron (A) solution	
7.	Rinse the section in Acetic acid, Colloidal Iron (A) solution	3 exchanges, 3 min each
8.	Add ≥5 drops of Potassium hexacyanoferrate, Colloidal Iron solution, and ≥5 drops of HCL reagent, Colloidal Iron. Note: Use fresh solution, discard after use.	20 min
9.	Rinse in distilled water	3 exchanges, 5 seconds each
10.	Stain using Fuchsin Acid Van Gieson reagent (add ≥5 drops)	30 seconds
11.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 5 dips each
12.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 2 min each
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 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

Blue - acid mucins
Hues of red - collagen
Yellow - muscle fiber, cytoplasm, erythrocytes, glial fibers

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 Colloidal Iron 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. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, 6th ed., Churchill Livingstone
2. Culling C.F.A., et all (1985): Cellular Pathology Technique, 4th ed., Butterworth-Heinemann
3. Mowry, R.W. (1963): The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcian blue g8x and their combinations with the periodic acid-schiff reaction, Ann NY Acad Sci , 106(2)
4. Nito Y., Stokes J.R. (1960) An improved colloidal iron staining reagent, Stain Technol, 103(35)

KOL-100T, V1-EN1, 21 August 2019, PL/IŠP

Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
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						