BIOGNOST®

VON KOSSA KIT

IVD *In vitro* diagnostic medical device CE

Five-reagent kit for calcium deposit and calcium salt staining

INSTRUCTIONS FOR USE

REF Catalogue number: VK-100T (100 tests) VK-K-100 (5 x 100 mL)

Introduction

Von Kossa kit is used for calcium deposits and calcium salt visualization. Silver ions from silver nitrate replace carbonate and phosphate calcium ions that, under strong source of light, in turn create microscopically visible silver glow. Treating the preparation with lithium carbonate prevents false positive staining from occurring. Counterstaining is achieved with Nuclear Fast Red (Kernechtrot) reagent.

Product description

- **VON KOSSA KIT**- Five-reagent kit for calcium deposit and calcium salt staining

The kit contains:	100 tests (VK-100T)	5 x 100 mL (VK-K-100)	Storage temperature
Lthium carbonate, solution	30 mL (LK-OT-30)	100 mL (LK-OT-100)	15-25°C
Silver ammonia reagent	30 mL (SAR-OT-30)	100 mL (SAR-OT-100)	2-8°
Reducing buffer, solution	30 mL (RP-OT-30)	100 mL (RP-OT-100)	15-25°C
Sodium thiosulfate, 5% solution	30 mL (NT5-OT-30)	100 mL (NT5-OT-100)	15-25°C
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	100 mL (KR-OT-100)	15-25°C

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

CAUTION:

Adhere to the following rules in order to achieve the best results:
- use distilled or demineralized high purity water **WITHOUT** any chlorine
- use completely clean laboratory glassware
- avoid contact between metal objects and solution (scissors, tweezers and so on)
- do not use sections fixed in fixatives that contain salts of heavy metals, such as mercury chloride and potassium dichromate because they may cause false positive reaction
- alkaline solutions during staining and may cause section dropping off the slide We suggest using positively charged glass slides

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.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests (VK-100T)

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Start rehydration by 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.	Treat the sections with Lithium carbonate, solution (add ≥5 drops)	10 min
6.	Rinse in distilled water	
7.	Treat Silver ammonia solution in dark room (add ≥5 drops)	60 min
8.	Rinse in distilled water	4 exchanges
9.	Treat the sections using Reducing buffer, solution until silver salt turns black (add ≥5 drops)	5 minutes or longer
10.	Rinse in distilled water	
11.	Treat the sections with Sodium thiosulfate, 5% solution (add ≥5 drops)	5 min
12.	Rinse in distilled water	
13.	Stain the section with Nuclear Fast Red (Kernechtrot) reagent (add ≥5 drops)	5 min

14.	Rinse under tap water	2 min
15.	Dehydrate using 70% alcohol (Histanol 70)	2 exchanges, 30 seconds each
16.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
17.	Dehydrate using 100% alcohol (Histanol 100)	30 seconds
18.	Dehydrate using 100% alcohol (Histanol 100)	2 min
19.	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.

b) using five 100 mL reagents (VK-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.	Start rehydration by 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 into Lithium carbonate, solution	10 min
6.	Rinse in distilled water	
7.	Immerse into Silver ammonia solution in dark room	60 min
8.	Rinse in distilled water	4 exchanges
9.	Immerse into Reducing buffer, solution until silver salt turns black	5 minutes or longer
10.	Rinse in distilled water	
11.	Immerse into Sodium thiosulfate, 5% solution	5 min
12.	Rinse in distilled water	
13.	Immerse into Nuclear Fast Red (Kernechtrot) reagent	5 min
14.	Rinse under tap water	2 min
15.	Dehydrate using 70% alcohol (Histanol 70)	2 exchanges, 30 seconds each
16.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
17.	Dehydrate using 100% alcohol (Histanol 100)	30 seconds
18.	Dehydrate using 100% alcohol (Histanol 100)	2 min
19.	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.

Result

Bones and calcium deposits – black
Nuclei and cytoplasms – pink-red

Note

Staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

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

Components of Von Kossa kit are kept under different storage conditions. Keep reagents dry, at temperature indicated on the label in a tightly sealed original packaging. 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, Churchill Livingstone, New York.
2. Prophet, Mills, Arrington, Sobin (1968), Laboratory methods in histotechnology. Stain methods of the Arm Forces Institute of Pathology, Washington D.C.

VK-X, V7-EN4, 06 March 2019, IŠP/VR:

Refer to the supplied documentation	Storage temperature range	Number of tests in package	Product code	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 number	Manufacturer		
For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile				