BIOGNOST®

MASSON FONTANA KIT

IVD In vitro diagnostic medical device CE

Six-reagent melanin and argentaffin granules staining kit
INSTRUCTIONS FOR USE

REF Catalog number: MF-100T (100 tests) MF-K-100 (9x100 mL) MF-K-500 (9x500 mL)

Introduction

Masson Fontana kit is used in a specific method for proving argentaffin granules in histological sections, based on reduction of silver nitrate to elemental silver. Melanin is pigment usually found in skin, hair, retina, and some parts of central nervous system. In order to avoid getting false positive results, BioGnost's Masson Fontana kit contains reagents for melanin depigmentation. Depigmentation is conducted on a control section before impregnation using silver.

Product description

MASSON FONTANA KIT - Six-reagent melanin and argentaffin granules staining kit.

The kit contains:	100 tests (MF-100T)	9 x100 mL (MF-K-100)	9 x500 mL (MF-K-500)	Storage temperature
Potassium permanganate, 0.5% solution	30 mL (KP05-OT-30)	100 mL (KP05-OT-100)	500 mL (KP05-OT-500)	15-25°C
Sulfuric acid, 0.5% solution	30 mL (SK05-OT-30)	100 mL (SK05-OT-100)	500 mL (SK05-OT-500)	15-25°C
Oxalic acid, 1% solution	30 mL (OKS1-OT-30)	100 mL (OKS1-OT-100)	500 mL (OKS1-OT-500)	15-25°C
Silver ammonia reagent	2x30 mL (SAR-OT-30)	2x100 mL (SAR-OT-100)	2x500 mL (SAR-OT-500)	2-8°C
Sodium thiosulfate, 5% solution	2x30 mL (NT5-OT-30)	2x100 mL (NT5-OT-100)	2x500 mL (NT5-OT-500)	15-25°C
Nuclear Fast Red (Kernechtrot) reagent	2x30 mL (KR-OT-30)	2x100 mL (KR-OT-100)	2x500 mL (KR-OT-500)	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 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 (double-filtered) 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 (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 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μm slices and mount them on **adhesive** VitroGnost glass slide.

Sample staining procedure

Note: Tissue samples must be mounted on VitroGnost adhesive glass slide in order to achieve optimal results (choose option a) in step 5**).** If that is not possible, choose **option b)** in step 5.

Note: Prepare two samples of the same tissue - one as a negative control section, the other for diagnosis assessment.

Melanin depigmentation will occur in **the negative control section** (using potassium permanganate and sulfuric acid).
On **the diagnosis assessment section** there will be no melanin depigmentation - the staining process is different and it begins from step 9. In the case of melanin occurrence in the sample, it will be visible (unlike the negative control section that lacks melanin).

PREPARING THE CONTROL SAMPLE – tissue whitening		
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.	**a) Tissue bleaching on adhesive glass slide:** 5 drops of Potassium permanganate, 0.5% solution + 5 drops of Sulfuric acid, 0.5% solution	5 min
	b) Tissue bleaching on regular glass slide: 5 drops of Potassium permanganate, 0.5% solution	30 min
6.	Rinse in distilled (demi) water	
7.	Treat with Oxalic acid, 1% solution	5 min
8.	Gently rinse in distilled (demi) water	
	Resume the procedure from step 13 (skip steps 9, 10, 11, 12)	

PREPARING THE SECTION FOR DIAGNOSIS ASSESSMENT		
9.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 10 min each
10.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
11.	Rehydrate using 95% alcohol (Histanol 95)	2 min
12.	Rehydrate in distilled (demi) water	2 min
13.	Place the sections in incubation container (Petri dish or transport box)	
14.	Treat the sections using Silver ammonia reagent. Discard the solution after using.	overnight at room temperature or for 30-40 minutes at 56°C
	Note: Let Silver ammonia reagent set for 10 min at room temperature before use	
15.	Rinse in distilled (demi) water	a few rinses
16.	Treat the sections with Sodium thiosulfate, 5% solution	1-2 minutes
17.	Rinse in distilled (demi) water	a few rinses
18.	Stain using Nuclear Fast Red (Kernechtrot) reagent	5 min
19.	Rinse in distilled (demi) water	
19.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
20.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
21.	Dehydrate using 100% alcohol (Histanol 100)	2 min
22.	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

Melanin, argentaffin granules - black (diagnosis assessment section); black color is not present in the control section because of the melanin depigmentation process
Nuclei - red

Note

Microbiology 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 Masson Fontana 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. Melis, M., Carpino, F., Di Tondo, U., Ermes, E. Tecniche in anatomia patologica. 1989.
2. Prophet, E.B., Mills, B., Arrington, J., Sobin, L. Laboratory methods in histotechnology. American Registry of Pathology.
3. Bancroft, J.D., Gamble, M. Livingstone, C. Theory and practice of Histological Techniques – 5° edizione 2002.

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