BIOGNOST®

MASSON FONTANA KIT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Six-reagent kit for staining melanin and argentaffin granules

INSTRUCTION FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalogue number**	**Volume**	**UDI-DI number**
MF-100T	100 tests	03858890002478
MF-K-100	9 x 100 mL	03858890004939
MF-K-500	9 x 500 mL	03858892122785

Intended use and test principle

The Masson Fontana kit is used in a specific method for demonstrating melanin and argentaffin granules in histological sections. In a positive argentaffin reaction, cells take up silver from the Silver Ammonium reagent and reduce it to a visible, metallic silver without the aid of a reductant. Melanin is a pigment normally found in the skin, hair, retina, and some parts of the central nervous system. To avoid the possibility of obtaining a false positive result, BioGnost's Masson Fontana kit contains reagents for melanin depigmentation. Depigmentation is carried out on the control slide before impregnation with silver.

Description of the product

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

The kit contains:	**100 tests (MF-100T)**	**9 x 100 mL (MF-K-100)**	**9 x 500 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 ammonium 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

Additional reagents and materials that can be used in the method

- Fixative agents such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion media such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 micron thin slices and mount on a VitroGnost microscope slide

NOTE

Apply the reagent to completely cover the section.

For optimal results, the tissue samples should be mounted on a VitroGnost adhesive glass slide (choose option "a" in step number 5). If this is not possible, select option "b" in step number 5.

Preparation of the slide:

Prepare two slides of the same sample tissue, one of which will be a negative control slide, and the other diagnostic slide.

- Melanin depigmentation is performed on the negative control slide using potassium permanganate and sulfuric acid.
- Melanin depigmentation is not performed on the diagnostic slide, the staining procedure is different and starts from step number 9. In this case, if there is melanin in the sample, it will be visible (unlike the negative control slide where there is no melanin).

Sample staining procedure

PREPARATION OF NEGATIVE CONTROL SLIDE – tissue bleaching		
1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 changes, 2 minutes each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 changes, lasting 5 and 3 minutes
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydrate in distilled/demineralized water	2 minutes
5.	**a) Bleaching of tissue on an adhesive glass slide:** 5 drops of potassium permanganate, 0.5% solution + 5 drops of sulfuric acid, 0.5% solution	5 minutes
	b) Bleaching of tissue on a normal glass slide: 5 drops of potassium permanganate, 0.5% solution	30 minutes
6.	Rinse in distilled/demineralized water	
7.	Treat with oxalic acid, 1% solution	5 minutes
8.	Rinse gently in distilled/demineralized water	
	Continue with step 13 (skip steps 9, 10, 11 and 12)	

PREPARATION OF DIAGNOSTIC SLIDE		
9.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 changes, 2 minutes each
10.	Rehydrate in 100% alcohol (Histanol 100)	2 changes, lasting 5 and 3 minutes
11.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
12.	Rehydrate in distilled/demineralized water	2 minutes
13.	Place the slides in the incubation container (Petri dish or transport box)	
14.	Treat slides with Silver ammonium reagent. Discard the solution after use.	overnight at room temperature or 30-40 minutes at 56°C
	Note: Leave the silver ammonium reagent at room temperature for 10 minutes before use	
15.	Rinse in distilled/demineralized water	several rinses
16.	Treat slides with sodium thiosulfate, 5% solution	1-2 minutes
17.	Rinse in distilled/demineralized water	several rinses
18.	Staining of slides with Nuclear Fast Red (Kernechtrot) reagent	5 minutes
19.	Rinse in distilled/demineralized water	
19.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
20.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
21.	Dehydrate in 100% alcohol (Histanol 100)	2 minutes
22.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 minutes each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

Result

Melanin and argentaffin granules - black color on the diagnostic slide, and black color is missing on the negative control slide because melanin depigmentation was performed
Nuclei - red color

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the described staining procedure may cause variations in results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, histological processing of samples and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control. If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry, well-closed original packaging at a temperature of +15 °C to +25 °C. Keep Silver ammonium reagent at a temperature of +2 °C to +8 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration date are printed on the product label.

Literature

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

Warnings and precautions regarding the materials contained in the product:	
	H412 Harmful to aquatic life with long lasting effects. P273 Avoid release to the environment. P501 Dispose of contents/container in accordance with local regulations.

MF-IFU, EN12, 02.07.2024, LO/IŠP

	Manufacturer	LOT	Batch code	°C °F	Temperature limit	IVD	*In vitro* diagnostic medical device	UDI	Unique device identifier
	Date of manufacture	REF	Catalogue number		Consult instructions for use	Σ	Contains sufficient for <n>tests		
	Use-by date		Fragile, handle with care		Caution	CE	European conformity		

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Version	Description / reason for change	Date
12.	Revised in acc. to Regulation (EU) 2017/746 - IVDR	02.07.2024.