BIOGNOST®

MARTIUS SCARLET BLUE (MSB) KIT

IVD *In vitro* diagnostic medical device CE

Seven-reagent kit for staining fibrins and collagen

INSTRUCTIONS FOR USE

REF Product code: MSB-100T (for 100 tests) MSB-K-100 (6 x 100 mL + 1 x 250 mL)

Introduction

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Martius Scarlet Blue (MSB) staining technique is used for fibrin visualization, especially of older clusters. This method is a modified Masson Trichrome method and it is ideal for studying connective tissue and vascular pathology.

Product description

- **MARTIUS SCARLET (MSB) BLUE KIT**– Seven-reagent kit for staining fibrins and collagen

The kit contains:	100 tests (MSB-100T)	6x100 mL + 1x250 mL (MSB-K-100)
Martius Yellow, solution	30 mL (MAY-OT-30)	100 mL (MAY-OT-100)
Ponceau S, solution	30 mL (PONS-OT-30)	100 mL (PONS-OT-100)
Aniline Blue, solution	30 ml (ABO-OT-30)	100 ml (ABO-OT-100)
Phosphotungstic acid, 1% solution	30 mL (FVK1-OT-30)	100 mL (FVK1-OT-100)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)
Ferri reagent, Weigert B	30 ml (FR-OT-30)	100 ml (FR-OT-100)
Acid alcohol	100 mL (KA-OT-30)	250 mL (KA-OT-250)

Preparing 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 (MSB-100T)

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.	Apply 5 drops of Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react	10 min
	Note: Working solution is stable for approximately 2 weeks. After the nuclei start turning brown, discard the working solution	
6.	Rinse the section quickly under tap water	
7.	Immerse into Acid alcohol, MSB	2 dips
8.	Rinse the section in tap water	5 min
9.	Rinse using 95% alcohol (Histanol 95)	2 min
10.	Add Martius Yellow solution (≥5 drops)	5 min
11.	Rinse in demineralized water	2 min
12.	Add Ponceau S solution (≥5 drops)	10 min
13.	Rinse in demineralized water	2 min
14.	Add Phosphotungstic acid, 1% solution (≥5 drops)	4 min
15.	Rinse in demineralized water	2 min
16.	Add Aniline Blue solution (≥5 drops)	5 min
17.	Rinse in demineralized water	2 min
18.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
19.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
20.	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.

b) using seven-reagent kit (MSB-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.	Prepare the Weigert hematoxylin working solution: mix equal volumes of Hematoxylin, Weigert A and Ferri reagent, Weigert B	10 min
	Note: Prepare the working solution of volume adequate for staining test sections. Working solution is stable for approximately 2 weeks. After the nuclei start turning brown, discard the working solution	
6.	Rinse the section quickly under tap water	
7.	Immerse into Acid alcohol, MSB	2 dips
8.	Rinse the section in tap water	5 min
9.	Rinse using 95% alcohol (Histanol 95)	2 min
10.	Immerse into Martius Yellow solution	5 min
11.	Rinse in demineralized water	2 min
12.	Immerse into Ponceau S solution	10 min
13.	Rinse in demineralized water	2 min
14.	Immerse into Phosphotungstic acid, 1% solution	4 min
15.	Rinse in demineralized water	2 min
16.	Immerse in Aniline Blue solution	5 min
17.	Rinse in demineralized water	2 min
18.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
19.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
21.	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

Nuclei - blue/black
Muscles - dark red
Fibrins - red (young clusters may be stained yellow, and older ones blue)
Collagen - blue
Erythrocytes - yellow

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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 Martius Scarlet Blue (MSB) kit in a tightly sealed original packaging at temperature of +15 to +25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. John, D. B. (2009): Theory and Practice of Histological Techniques, 6th ed.,
2. Stefan, J. A. (1984): Simultaneous demonstration of connective tissue elastica and fibrin by a combined Verhoeff 's elastic - Martius - Scarlet - Blue trichrome stain, Stain Technology, 59(1): 1-5
3. Mariusz, G. et al. (2017): Combined orcein and martius scarlet blue (OMSB) staining for qualitative and quantitative analyses of athrosclerotic plaques in brachiocephalic arteries in apoE/LDLR $^{-/-}$ mice, Cross Mark, 147(6): 671-681
4. Lendrum, A. C. (1962): Studies on the character and staining of fibrin, J Clin Pathol., 15(5): 401-413

MSB-X, V1-EN1, 3 September 2019, LS/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						