BIOGNOST®

MASSON-GOLDNER TRICHROME KIT

NEW STAINING PROCEDURE

IVD *In vitro* diagnostic medical device CE

Seven-reagent muscle and collagen fiber staining kit

INSTRUCTIONS FOR USE

REF Catalogue number: MGT-100T (100 tests) MGT-K-100 (7x100 mL) MGT-K-500 (7x500 mL)

Introduction

Masson-Goldner Trichrome kit is used for visualization of muscles, collagen fibers, connective tissues, gametes, nuclei, neurofibrils, neuroglia, collagen, keratin intracellular fibrils and negative visualization of the Golgi apparatus. Method of staining muscle and collagen fibers in tissues during which Fast Green F.C.F. dye binds with collagen making it turn distinct green. It is also used for visualization of increased collagen build up associated with functioning tissue being mistaken for scar tissue (liver sclerosis diagnosis), but also for differentiating smooth muscle fibers and collagens.

Product description

- **MASSON-GOLDNER TRICHROME KIT** - Seven-reagent muscle and collagen fiber staining kit

The kit contains:	100 tests (MGT-100T)	7 x 100 mL (MGT-K-100)	7 x 500 mL (MGT-K-500)
Bouin's solution	100 mL (BOU-OT-100)	100 mL (BOU-OT-100)	500 mL (BOU-OT-500)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)	500 mL (HEMA-OT-500)
Ferri reagent, Weigert B	30 ml (FR-OT-30)	100 ml (FR-OT-100)	500 mL (FR-OT-500)
Biebrich Scarlet-Acid Fuchsin reagent	30 ml (BSAF-OT-30)	100 ml (BSAF-OT-100)	500 mL (BSAF-OT-500)
P.T.A.-P.M.A. reagent	30 mL (PPR-OT-30)	100 mL (PPR-OT-100)	500 mL (PPR-OT-500)
Fast Green F.C.F. reagent	30 mL (FGR-OT-30)	100 mL (FGR-OT-100)	500 mL (FGR-OT-500)
Acetic acid, 1% solution	30 mL (OK1-OT-30)	100 mL (OK1-OT-100)	500 mL (OK1-OT-500)

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 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

Preparing the histological sections for staining

- Fixate 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.

NOTE: Apply the reagent so it completely covers the section.

Sample staining procedure - NEW STAINING PROCEDURE!

a) using kit for 100 tests (MGT-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.	Stain using Bouin's solution	60 min at 56˚C or over night at room temperature
6.	Cool the section down at room temperature	10 min
7.	Rinse under tap water	10 seconds
8.	Rinse in distilled water	10 seconds
9.	Stain using Hematoxylin, Weigert A (5 drops) and Ferri reagent, Weigert B (5 drops)	15-20 min
10.	Rinse under tap water	3 min
11.	Stain using Biebrich Scarlet-Acid Fuchsin reagent (10 drops)	20 min
12.	Rinse in distilled water	until the excessive dye is washed off of the section
13.	Treat using PTA-PMA reagent (10 drops)	10 min
14.	Pour the reagent off the section without rinsing	
15.	Stain using Fast Green F.C.F. reagent (10 drops)	5-7 min
16.	Rinse in distilled water	until the excessive dye is washed off of the section
17.	Treating using 1% acetic acid solution (10 drops)	1-2 seconds
18.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate using 100% alcohol (Histanol 100)	2 min
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 a VitroGnost cover glass.

b) using seven-reagent 100 mL or 500 ml kit (MGT-K-100, MGT-K-500)

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.	Immerse into Bouin's solution	60 min at 56˚C or over night at room temperature
6.	Cool the section down at room temperature	10 min
7.	Rinse under tap water	10 seconds
8.	Rinse in distilled water	10 seconds
9.	Prepare Weigert hematoxylin working solution: mix equal volumes of Hematoxylin, Weigert A and Ferri reagent, Weigert B	
	Note: working solution is stable for approximately 2 weeks. Prepare the working solution of volume adequate for staining test sections	
10.	Immerse into Weigert hematoxylin working solution and let it react	15-20 min
11.	Rinse under tap water	3 min
12.	Immerse into Biebrich Scarlet-Acid Fuchsin reagent	20 min
13.	Rinse in distilled water	until the excessive dye is washed off of the section
14.	Immerse into PTA-PMA reagent	10 min
15.	Pour the reagent off the section without rinsing	
16.	Immerse into Fast Green F.C.F. reagent	5-7 min
17.	Rinse in distilled water	until the excessive dye is washed off of the section
18.	Immerse into 1% acetic acid solution	1-2 dips
19.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
20.	Dehydrate using 100% alcohol (Histanol 100)	2 min
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 a VitroGnost cover glass.

Results

Nuclei – blue-purple
Muscle fibers, keratin, cytoplasm - bright red
Collagen, mucus - green
Erythrocytes - red-orange

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

Keep Masson-Goldner Trichrome 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. Melis, M., Carpino, F., Di Tondo, U. (1989), Tecniche in anatomia patologica, Edi Ermes, Milano.
2. Prophet, E.B., Mills, B., Arrington, J., Sobin, L. (1968), Laboratory methods in histotechnology, McGraw Hill, Washington D.C.
3. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.

MGT-X, V8-EN8, 27 March 2020, IŠP/VR

	Refer to the supplied documentation	°C	Storage temperature range	Σ	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						