BIOGNOST®

LIGHT GREEN S.F. REAGENT

IVD In vitro diagnostic medical device CE

Counterstain used with trichrome special staining kits

INSTRUCTIONS FOR USE

REF Product code: LGS-OT-100 (100 mL)

Introduction

Light Green S.F. reagent is used in special staining kits for staining muscle and collagen fibers in tissues during which Light Green S.F. dye binds with collagen making it turn distinct green.

Product description

- **LIGHT GREEN S.F. REAGENT** – green counterstain for use with special staining kits

Other slides 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, BioClear New agent on the aliphatic hydrocarbons basis.
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR, or one of 30 (and more) BioGnost's glass slides.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam or MountQuick Tube medium
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion oils, such as BioGnost's Immersion oil, Cedarwood oil, Immersion oils types 37, A, B, FF and NVH

Preparing the histological sections for staining

- Fixate 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 place them on a VitroGnost glass slide

Example of a staining procedure using Light Green S.F. reagent (Masson-Goldner trichrome stain)

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 10 min each
2.	Rehydration using 100% alcohol (Histanol 100)	2 exchanges in, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Staining using Bouin's solution	60 min at 56˚C or overnight at room temperature
6.	Cool the section down at room temperature	10 min
7.	Rinse under tap water	10 seconds
8.	Rinsing in distilled water	10 seconds
9.	Staining using Hematoxylin, Weigert A (5 drops) and Ferri reagent, Weigert B (5 drops)	5 min
10.	Rinse under tap water	3 min
11.	Staining using Biebrich Scarlet-Acid Fuchsin reagent (10 drops)	2 min
12.	Rinsing in distilled water	until the excessive dye is washed off of the section
13.	Treating using P.T.A.-P.M.A. reagent (10 drops)	10 min
14.	Pour the reagent off the section without rinsing	
15.	Staining using Light Green S.F. reagent (10 drops)	5 min
16.	Rinsing in distilled water	until the excessive dye is washed off of the section
17.	Treating using 1% acetic acid solution (10 drops)	3 min
18.	Dehydration using 70% alcohol (Histanol 70)	5 dips
19.	Dehydration using 95% alcohol (Histanol 95)	5 dips
20.	Dehydration using 100% alcohol (Histanol 100)	2 min
21.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 5 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

Nuclei - black
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 Light Green S.F. reagent 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.

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	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						