LITHIUM CARBONATE, LUXOL

IVD *In vitro* diagnostic medical device CE

For use in Luxol Fast Blue kit

INSTRUCTIONS FOR USE

REF Catalogue number: LKL-OT-100 (100 mL)

Introduction

Lithium carbonate, Luxol is a part of Luxol Fast Blue kit (acc. to Kluewer-Barrera) which is used for detecting myelin and Nissl bodies on histological sections and for visualizing basic structure of brain tissue and spinal cord tissue.

Product description

- **LITHIUM CARBONATE, LUXOL** – Lithium carbonate aqueous solution

Example of using Lithium carbonate, Luxol in Luxol Fast Blue kit

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 52/54, BioWax 56/68, BioWax Blue
- 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
- High-quality adhesive glass slides for use in histopathology and cytology, such as VitroGnost adhesive 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
- Other components of Luxol Fast Blue kit: Luxol Fast Blue, solution (LFB-OT-100) and Cresyl Violet, solution (CV-OT-100)

Preparing the histological sections for staining

- Fix the tissue sample well (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).
- Cut the paraffin block to **5-7 µm** slices and place them on a VitroGnost adhesive glass slide.

Sample staining procedure

Note: drip the reagents onto the section and cover them completely. In order to avoid evaporation of the solutions, use incubation (for instance, Petri) dishes. If necessary, add more solution if evaporation occurs.

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 10 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.	Stain with Luxol Fast Blue, solution	overnight at 37°C or for 2 hours at 60°C
5.	Rinse in 95% alcohol (Histanol 95) until formed crystals dissolve	several rapid dips
6.	Rinse in distilled (demi) water	
7.	Treat with lithium carbonate solution, Luxol	5-30 seconds
	Note: use the microscope in order to check if the grey matter differs from white matter, repeat this step if necessary	
8.	Immerse the section into 70% ethyl alcohol (Histanol 70) and let it set until myelin fibers turn blue on a transparent background (check using microscope).	several rapid dips
9.	Rinse thoroughly in distilled (demi) water twice	several dips
10.	Add 10 drops of Cresyl Violet, solution	30-60 minutes at 60 °C
11.	Immerse the section into 95% ethyl alcohol (Histanol 95) and let it set until Nissl bodies turn light pink	Several quick dips
12.	Dehydrate using 100% alcohol (Histanol 100)	2 min
13.	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.

Results

Myelin - turquoise blue
Neurons and glia cells nuclei - pink to purple
Nissl bodies - pale pink

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 Lithium carbonate, Luxol solution in a tightly closed original package at temperature of +15°C 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. Kluver et Barrera (1953), A method for the combined staining cells and fibres of nervous system, J Neuropathol and Exp Neurology, 49:67-69
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		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						