

# LUXOL FAST BLUE, SOLUTION

IVD *In vitro* diagnostic medical device CE

For use with Luxol Fast Blue kit

## INSTRUCTIONS FOR USE

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

### Introduction

Luxol Fast Blue, solution is a component of Luxol Fast Blue kit used for visualization of myelin, Nissl bodies on histology sections, and for displaying basic structure of brain tissue and spinal cord tissue (acc. to Kluewer-Barrera).

### Product description

- **LUXOL FAST BLUE, SOLUTION** – Luxol Fast Blue alcoholic solution with added stabilizer.

### Example of staining with Luxol Fast Blue, solution as a component of Luxol Fast Blue kit

#### Other sections and reagents necessary for 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, 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 DPX Low Eco, 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
- BioGnost's reagents that comprise Luxol Fast Blue kit: Lithium carbonate, Luxol (product code LKL-OT-100), and Cresyl Violet, solution (product code CV-OT-100)

### Preparing the 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  $\mu$ m slices and place them on a VitroGnost 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 VitroGnost cover glass.

## Result

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 Luxol Fast Blue, solution in a tightly closed original package at room temperature. 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.

LFB-OT-100, V1-EN1, 17 April 2019, IŠP/VR

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity	 BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA <a href="http://www.biognost.com">www.biognost.com</a>	
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer		
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile						