

LUXOL FAST BLUE KIT

IVD In vitro diagnostic medical device



Three-reagent myelin and phospholipid staining kit

INSTRUCTIONS FOR USE

REF Catalogue number: LFB-100T (100 tests) LFB-K-100 (3x100 mL)

Introduction

Luxol Fast Blue kit (acc. to Kluewer-Barrera) 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

- **LUXOL FAST BLUE KIT** - Three-reagent myelin and phospholipid staining kit

| The kit contains: | 100 tests (LFB-100T) | 3x 100 mL (LFB-K-100) |
|---------------------------|----------------------|-----------------------|
| Luxol Fast Blue, solution | 30 mL (LFB-OT-30) | 100 mL (LFB-OT-100) |
| Lithium carbonate, Luxol | 30 mL (LKL-OT-30) | 100 mL (LKL-OT-100) |
| Cresyl Violet, solution | 30 mL (CV-OT-30) | 100 mL (CV-OT-100) |

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, 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

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 **5-7 µm** 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 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 Luxol Fast Blue kit 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-X, V6-EN5, 11 July 2023, LO/IŠP

| | | | | | | | | | |
|---|---|---|----------------------------------|---|----------------------------|---|--------------|---|---------------------|
|  | Refer to the supplied documentation |  | Storage temperature range |  | Number of tests in package |  | Product code |  | European Conformity |
|  | 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 | | | | |



BIOGNOST Ltd.
Medjugorska 59
10040 Zagreb
CROATIA
www.biognost.com

