BIOGNOST®

LUXOL FAST BLUE KIT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Three-reagent kit for staining myelins and phospholipids

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
LFB-100T	For 100 tests	03858890002065
LFB-K-100	3x100 mL	03858892120453

Intended use and test principle

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. Luxol Fast Blue method enables selective staining of myelin sheaths in nerve tissue. Luxol Fast Blue solution, an acid soluble in lipids, binds to lipoprotein components of myelin, creating a stable blue complex. Lithium carbonate acts as a differentiating agent, removing excessive dye from surrounding tissue providing a stronger contrast between stained myelin and background. Cresyl Violet solution is used to counterstain nuclei, enabling a clear identification of neural and glial structures. The result is intense blue myelin, purple nuclei and pale surrounding tissue, providing a clear visualization of white matter.

Product description

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

The kit contains:	**100 tests (LFB-100T)**	**3 x 100 mL (LFB-K-100)**	**Storage emperature**
Luxol Fast Blue, solution	30 mL (LFB-OT-30)	100 mL (LFB-OT-100)	15-25°C
Lithium carbonate, Luxol	30 mL (LKL-OT-30)	100 mL (LKL-OT-100)	15-25°C
Cresyl Violet, solution	30 mL (CV-OT-30)	100 mL (CV-OT-100)	15-25°C

Additional reagents and materials that can be used in this method

- 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, 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 DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 μm thin slices and mount on a VitroGnost microscope slide

NOTE

Apply the reagent so that it completely covers the section. To avoid solution evaporation, use incubation dishes (e.g. Petri dishes). If evaporation occurs, add more drops of solution if necessary.

Histological sample staining procedure

a) using kit for 100 tests (LFB-100T)

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

b) using three-reagent kit, 100 mL (LFB-K-100)

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 in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 10 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min

4.	Immerse into 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 quick dips
6.	Rinse in distilled/demineralized water	
7.	Immerse into Lithium carbonate, Luxol, solution	5-30 sec
	Note: use the microscope in order to check if the grey matter differs from white matter, repeat this step if necessary	
8.	Immerse the slide into 70% ethyl alcohol (Histanol 70) and let it set until myelin fibers turn blue on a transparent background (check using microscope)	several quick dips
9.	Rinse thoroughly in distilled /demineralized water twice	several dips
10.	Immerse into Cresyl Violet, solution	30-60 min at 60°C
11.	Immerse into 95% alcohol (Histanol 95) and let it set until Nissl bodies become pale pink	several quick dips
12.	Dehydrate in 100% alcohol (Histanol 100)	2 minute
13.	Clear in xylene (BioClear) or 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 – turqoise blue
Neurons and glial cells nuclei – pink to purple
Nissl bodies – pale pink

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.
If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product 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

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapor.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

LFB-IFU_ENV6, 25.02.2026., IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	Σ	Contains sufficient for <n>tests
	Date of manufacture	REF	Catalogue number		Caution	CE	European conformity
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device	UDI	Unique device identifier

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Version	Description / reason for change	Date
6	Revised acc. to Regulation (EU) 2017/746 - IVDR	25.02.2026.