BIOGNOST®

LEUKOGNOST PERLS

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

LeukoGnost PERLS (Prussian blue) kit for detection of free ferric (Fe3+) ions in cells

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30702CYTOS9	
EMDN code	W01030702	
REF **Catalog number**	**Volume**	**UDI-DI broj number**
LKG-PERLS	for 100 tests	03858892124451

Intended use and test principle

LeukoGnost PERLS kit is used for detection of free ferric ions (Fe3+) (not bound to hemoglobin) in cells, especially in normoblasts (sideroblasts), macrophages (hemosiderin), and other cells containing free iron. The method is based on Prussian/Berlin blue reaction and it is used for staining blood smears and bone marrow smears, as well as bone marrow histology sections. Prussian blue derives its name from the German pathologist Max Perls, who described the technique. The staining method is based on the reaction of ferric ion (Fe3+), not bound to hemoglobin with potassium hexacynoferrate (II) in HCl – solution creating a blue non – soluble sediment (salt complex).

$$4\ Fe^{3+} + 3\ K_4Fe(CN)_6 = Fe_4[Fe(CN)_6]_3 + 12\ K^+$$

In order to achieve the best possible visual differentiation of ferric deposits in cytoplasm, Nuclear Fast Red reagent is used as counterstain - it stains the nuclei red. LeukoGnost PERLS kit is used for diagnosing myelodysplastic syndrome that includes refractory anemia and chronic myelomonocytic leukemia. In case of anemias, 15% of red blood cells in bone marrow comprise of sideroblasts that contain at least 5 hemosiderin granules that get stained with Berlin blue. Ferric granules can be distributed in cytoplasm diffusely or perinuclearly (ring-shaped sideroblasts).

Product description

- **LEUKOGNOST PERLS** –kit for the detection of free ferric ions in cells

The kit contains:	**LKG-PERLS (for 100 tests)**	**Storage temperature:**
Reagent 1 (Potassium hexacyanoferrate, solution)	100 mL (KHC-OT-100)	15-25 ˚C
Reagent 2 (HCL reagent, LeukoGnost Perls)	100 mL (HCLL-OT-100)	15-25 ˚C
Reagent 3 (Nuclear Fast Red reagent)	2 x 100 mL (KR-OT-100)	15-25 ˚C

Additional reagents and materials that can be used in this method

- Methyl alcohol for fixing sections, such as Histanol M
- Water-based covering medium for microscope slides and mounting medium for cover glasses, such as BioGnost's BioMount Aqua medium (BMA-30).
- BioGnost's immersion media, such as Immersion oil (IU-30) or Immersion oil type A (IUA-30)
- VitroGnost slides and coverslips for use in histopathology and cytology
- 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, BioWax 56/68, BioWax Blue,

Preparing the staining solution

- In a clean tube mix Potassium hexacyanoferrate, solution and HCl reagent, LeukoGnost Perls in 1:1 ratio.
  Prepare fresh staining solution before each staining.

Adjust the reagent volume accordingly:

REAGENT	FOR 1 SLIDE	FOR 100 SLIDES
Reagent 1 (Potassium hexacyanoferrate, solution)	1 mL	100 mL
Reagent 2 (HCL reagent, LeukoGnost Perls)	1 mL	100 mL

NOTE

Apply the reagent so it completely covers the sample.

Preparing the smear for staining

- Whole blood or bone marrow smear should be thin and dry (dry the smear for at least 30 min). Such smears must not be older than three days.
- Fix the smear using the following method:

1.	Fix the dried smear in methanol (Histanol M)	3 min
2.	Dry the smear	

A) Blood smears and bone marrow smears staining procedure

1.	Apply the staining solution to the sample, 2 mL	20 min
2.	Carefully rinse in distilled water	
3.	Stain the smear with Reagent 3 (Nuclear Fast Red reagent), 2 mL	5 min
4.	Rinse in distilled water	
5.	Dry the smear	

After the smear is dried, it is recommended to mount the cover slide using BioMount Aqua medium to preserve color and sample quality.

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

B) Histology sections staining procedure

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 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.	Rehydrate in distilled/demineralized water	2 min
5.	Apply staining solution to the sample, 2 mL	20 min
6.	Rinse carefully in distilled/demineralized water	
7.	Stain with Reagent 3 (Nuclear Fast Red reagent), 2 mL	5 min
8.	Rinse in distilled/demineralized water *	
9.	Dehydrate in 70% alcohol (Histanol 70)	2 exchanges, 1 min each
10.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 1 min each
11.	Dehydrate in 100% alcohol (Histanol 100)	2 exchanges, 1 min each
12.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the slide. 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 slide with VitroGnost cover glass.
* It is possible to use water-based BioMount Aqua mounting medium for cover glasses immediately after final rinsing in distilled water and the slide drying. In that case dehydration and clearing are not necessary.

Result

Free ferric ions (Fe^{3+}) – granules stained blue
Nuclei – red
Cytoplasm – 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. Dharwadkar, A. et al. (2016): Study of sideroblasts and iron stores in bone marrow aspirates using Perls' stain, Medical Journal of Dr. D.Y. Patil University, str. 181-185.
2. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2 ed ed., Butterworth, London, UK.
3. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, str. 14-167.

Warnings and precautions regarding the materials contained in the product:
Not a hazardous substance or mixture acc. to Regulation (EZ) br. 1272/2008.

LKG-PERLS-IFU_ENV3, 19.12.2025., 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

BioGnost Ltd.
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
3	Revised in acc. to Regulation (EU) 2017/746 - IVDR	19.12.2025.