LEUKOGNOST PERLS

IVD *In vitro* diagnostic medical device CE

LeukoGnost PERLS (Prussian blue) kit for detection of free ferric (Fe^{3+}) ions in cells

INSTRUCTIONS FOR USE

REF Product code: LKG-PERLS (for 100 tests)

Introduction

LeukoGnost PERLS kit is used for detection of free ferric ions (Fe^{3+}) (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. Perls Prussian blue derives its name from the German pathologist Max Perls who described the technique. The method of staining is based on the reaction of ferric ion (Fe^{3+}), not bound to hemoglobin with potassium hexacyanoferrate (II) in HCl-solution creating 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** – three-reagent 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

Other sections and reagents that may be used with the procedure:

- 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)**
- 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
- 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 56/68, BioWax Blue, BioWax Micro.

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 SECTION	FOR 100 SECTIONS
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 section.

Preparing the section for staining

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

1.	Fix the sample in methanol (Histanol M)	3 min
2.	Dry the section	

A) Blood smears and bone marrow smears staining procedure

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

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

Preparing the histology sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), 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 Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

B) Histology sections staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 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.	Rehydrate in distilled (demi) water	2 min
5.	Apply the staining solution on the section, 2 mL	20 min
6.	Carefully rinse in distilled water	
7.	Stain the section with Reagent 3 (Nuclear Fast Red reagent), 2 mL	5 min
8.	Rinse in distilled water	
9.	Dehydrate using 70% alcohol (Histanol 70)	2 exchanges, 1 min each
10.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 1 min each
11.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
12.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 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.

NOTE

It is possible to use water-based BioMount Aqua mounting medium for cover glasses immediately after final rinsing in distilled water and the section drying. In that case dehydration and clearing are not necessary.

Result

Free ferric ions (Fe^{3+}) - granules stained blue
Nuclei - red
Cytoplasm - pink

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 LeukoGnost PERLS kit in a tightly sealed original packaging at temperature of +15 to +25°C. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's 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, pp. 181-185.
2. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2nd 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, 14-167.

LKG-PERLS, V3-EN2, 31 January 2023, SB/IŠP

Refer to the supplied documentation	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				