BIOGNOST®

LEUKOGNOST PLUS

IVD *In vitro* diagnostic medical device CE

Additional set of reagents for use with LeukoGnost kits

INSTRUCTIONS FOR USE

REF Product code: LKG-PLUS (for at least 100 tests)

Introduction

For adequate enzymatic activity of leukocytes of the whole blood, bone marrow or other cytology materials, it is necessary to properly fix the preparations. **LeukoGnost Fixative** reagent is used for fixing biological materials that can later be used in various cytochemical methods, including BioGnost's kits for diagnosing leukemia, available in LeukoGnost range. The fixative enables optimal enzymatic activity preservation, relevant for setting adequate clinical diagnosis.

BioGnost's **LeukoGnost HEM** is hematoxylin used as a dye for counterstaining cell nuclei. Progressive staining with kits from LeukoGnost range for cytochemical leukemia diagnostic is recommended. LeukoGnost HEM does not interfere with specific coloration that occurs during staining. LeukoGnost HEM is a highly stable hematoxylin and one of the hematoxylin formulations used in histopathology and cytology for a more precise nuclear cell staining. Cell nuclei are stained intense dark blue during staining blood and bone marrow sections. Hematoxylin is extracted from logwood (*Haematoxylon campechianum L.*). Hematoxylin is oxidized to hematein and chelates with metal ions (mordants), hematein turns into irreplaceable nuclear dye. Positively charged hematein-mordant complex then binds with negatively charged DNA phosphate ions, creating characteristic blue nuclear coloration. LeukoGnost HEM is 50% oxidized hematoxylin, with added aluminum as mordant and glycols as stabilizers.

BioGnost's **BioMount Aqua** is a water-based medium for covering microscope sections and mounting cover glasses. It provides the expected section transparency by using refractive index similar to refractive index of cover glasses and glass slides; this way the unwanted light refraction is avoided by providing clear and detailed image of the section. It is used for processing sections tested for enzymes and lipids, i.e. for testing samples that must not be dehydrated through series of ascending alcohol solutions and be cleared using xylene and xylene substitutes.

Product description

LeukoGnost PLUS – set of additional reagents for LeukoGnost kits

The kit contains:	LKG-PLUS (for 100 tests)	Storage temperature:
LeukoGnost Fixative	LKF-500 (500 mL)	15-25 °C
LeukoGnost HEM	LKH-OT-500 (500 mL)	15-25 °C
BioMount Aqua	BMA-30 (30 mL)	15-25 °C

- **LEUKOGNOST FIXATIVE** – fixative based on acetone and formalin, suitable for fixing blood and bone marrow smears
- **LEUKOGNOST HEM** – hematoxylin for use with LeukoGnost kits

BIOMOUNT AQUA - aqueous covering medium for covering microscope sections and mounting medium for cover glasses, pH 7.0, average viscosity of 200 cSt, and refractive index n_D=1.390-1.410 at +20 °C

Reagents that can be used with LeukoGnost PLUS kit:

- BioGnost's LeukoGnost range kits used for detecting and classifying leukemia: **LeukoGnost MPO** (kit for detecting myeloperoxidase activity in leukocytes), **LeukoGnost ALP** (kit for detecting alkaline phosphatase activity in leukocytes), **LeukoGnost ACP** (kit for detecting acid phosphatase activity in leukocytes), **LeukoGnost NSE** (kit for detecting non-specific esterase activity in leukocytes), **LeukoGnost SPE** (kit for detecting specific esterase activity in leukocytes), **LeukoGnost PAS** (kit for detecting periodic acid and Schiff 's reagent reaction in leukocytes), **and LeukoGnost SPENSE** (kit for simultaneous detection of specific and non-specific esterase activities in leukocytes).
- 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.

Procedure

Preparing the sample for staining

Fresh whole blood smears, bone marrow smears or precipitate of centrifuged sample are used as basic fixing materials. EDTA as anticoagulant is not recommended due to its interaction with enzymes that can subsequently result in weaker section staining using LeukoGnost range leukemia diagnostic kits.

Fixing procedure

1.	Fix the smear by applying LeukoGnost Fixative (1-2 mL) onto the slide	1-3 minutes
2.	Rinse the slide in distilled water	10 seconds
3.	Air dry the preparation and continue with staining with LeukoGnost kits	

Section staining procedure

Conduct staining according to instructions for use for the corresponding kit from LeukoGnost range for diagnosing leukemia.

Mounting the cover glass on the section

After staining and rinsing, dry the preparation in the appropriate manner. Using a dropper, add 1 drop of BioMount Aqua on a fixed glass slide. Carefully place a clean cover glass in order to avoid air bubbles. Press the cover glass so the layer of BioMount Aqua between two slides is as thin as possible and to remove residual air bubbles. Leave the glass slide in a horizontal position until the sample dries. It takes 10 minutes for BioMount Aqua to dry and harden.

LeukoGnost HEM staining result

Nuclei - blue

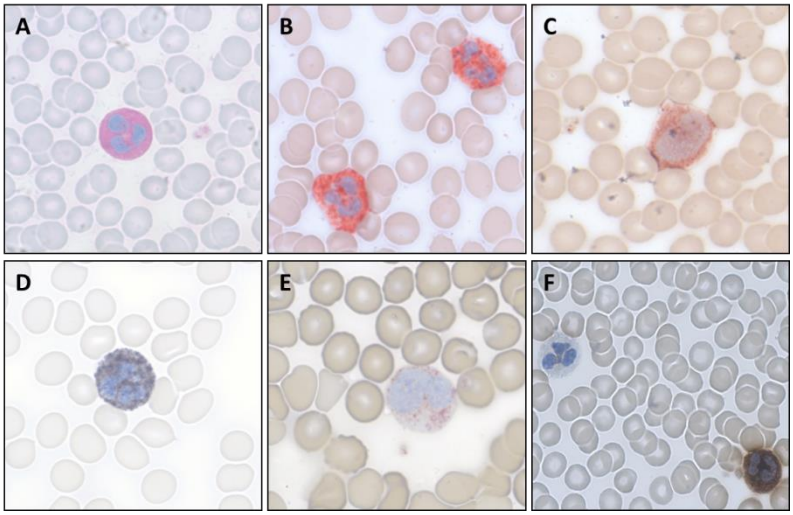

Figure 1 *Blood smears stained with* ***LeukoGnost PAS*** *(A),* ***LeukoGnost SPE*** *(B),* ***LeukoGnost NSE*** *(C),* ***LeukoGnost MPO*** *(D),* ***LeukoGnost ACP*** *(E) and* ***LeukoGnost ALP*** *(F) kits. Neutrophils (A, B, D and F) and monocytes (C and E) are shown. LeukoGnost Fixative was used for fixation, and LeukoGnost HEM was used for nuclear counterstaining. Magnification level is 1000x.*

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

Store LeukoGnost PLUS kit's reagents in a tightly closed original packaging at temperature between +15 °C and +25 °C. 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

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CE	European Conformity	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile		Manufacturer

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