

LEUKOGNOST PAS

IVD *In vitro* diagnostic medical device



Kit for detection of periodic acid-Schiff reaction in leukocytes

INSTRUCTIONS FOR USE

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

Introduction

LeukoGnost PAS kit contains reagents for cytochemical diagnosis of leukemia using bone marrow or whole blood samples. This staining method is based on the oxidation of intracellular glycogen and neutral mucopolysaccharides in the presence of periodic acid and Schiff's reagent. Periodic acid cleaves molecules containing glycol groups to create aldehydes which are affected by the Schiff's reagent in a reaction which stains the cytoplasm magenta. The kit is intended for individual testing of horizontally placed slides and it contains reagents for at least 100 tests for detecting periodic acid-Schiff reagent in leukocytes. The reagents are applied by dripping until the entire slide is covered (1-2 mL).

Product description

- **LEUKOGNOST PAS** – kit for detection of the periodic acid-Schiff reaction in leukocytes

The kit contains:	LKG-PAS (for 100 tests)	Storage temperature:
Reagent 1 (Periodic acid, LeukoGnost)	PKL-OT-100 (2x100 mL)	15-25 °C
Reagent 2 (Sodium metabisulfite, LeukoGnost)	NMLKG-OT-10 (2x10 mL)	15-25 °C
Reagent 3 (HCL reagent, LeukoGnost)	HCLLP-OT-10 (2x10 mL)	15-25 °C
Reagent 4 (BioSchiff Forte reagent)	BSF-OT-100 (2x100 mL)	15-25 °C (2-8 °C after first opening)

Other reagents necessary for the staining method

- **LeukoGnost Fixative (LKF-500)** – fixative for use in cytochemical diagnosis of leukemia
- **LeukoGnost HEM (LKF-OT-500)** – hematoxylin for use in cytochemical diagnosis of leukemia

or

- **LeukoGnost PLUS (LKG-PLUS)** – set of additional reagents for LeukoGnost kits

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

- 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 oils, such as **Immersion oil (IU-30)** or **Immersion oil type A (IUA-30)**

Preparation of sulfite solution:

- step 1: mix Reagent 2 and Reagent 3 in a clean tube
- step 2: add distilled water to mixture of Reagents 2 and 3

Note: Prepare the sulfite solution shortly before using. Modify the volume of the prepared sulfite solution according to the number of slides.

Modify the reagents' volume as necessary:

STEP	REAGENT	FOR 1 SECTION	FOR 12 SECTIONS	FOR 24 SECTIONS
step 1	reagent 2	200 µL (4 drops)	2.4 mL	4.8 mL
	reagent 3	200 µL (4 drops)	2.4 mL	4.8 mL
step 2	distilled water	4 mL	48 mL	96 mL

Preparing the section for staining

- Prepare the whole blood smear or bone marrow smear to be thin and dry (dry the smears for at least 30 mins). These sections must not be older than 3 days. Using anticoagulants is not recommended because it can inhibit the reaction.
- Fix the section the following way:

1.	Fix the smear by applying LeukoGnost Fixative (1-2 mL) onto the slide	1-3 minutes
2.	Rinse the slide under tap water	10 seconds
3.	Dry the preparation	

- Sections prepared and fixed in this manner can be stored at 2 to 8 °C and used for 3 days at most.

NOTE

Apply the reagent so it completely covers the slide.

The bottle containing BioSchiff Forte must be tightly closed in order to avoid SO₂ evaporation and to maintain the quality of the reagent. BioSchiff Forte is colorless or slightly orange solution. If it turns pink, it is no longer suitable for use.

Sample staining procedure

1.	Apply Reagent 1 (1-2 mL) onto the slide	10 min
2.	Rinse the slide in tap water	10 seconds
3.	Apply sulfite solution 1 (1-2 mL) onto the slide	1 min
4.	Apply Reagent 4 (1-2 mL) onto the slide	15 min
5.	Rinse the slide in tap water	10 seconds
6.	Apply sulfite solution 1 (1-2 mL) onto the slide	3 min
7.	Rinse the slide in tap water	3 min
8.	Stain the slide using LeukoGnost HEM reagent	3 min
9.	Rinse the slide under tap water	3 min
10.	Dry the preparation	

After drying the preparation, it is recommended to mount covering glass using BioMount Aqua medium and store in dark to preserve the color and quality of the sample.

Result

Promyelocytes, monocytes, basophils, neutrophils, megakaryocytes, platelets, 10-40% of lymphocytes - diffused to fine grainy magenta staining
Lymphoblasts, erythroblasts - cytoplasm magenta staining in form of large granules/blocks
Normal myeloblasts, eosinophils and erythrocytes - no specific staining

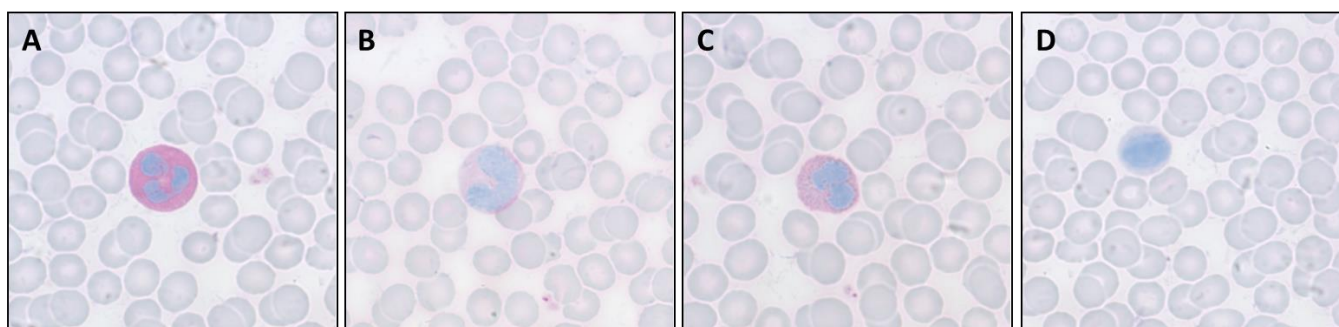


Figure 1. Blood smears stained with LeukoGnost PAS kit. Specific staining of neutrophils (A), monocyte (B) and basophil (C), and non-stained lymphocyte (D). Magnification level is 1000x.

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 PAS kit's reagents in a tightly closed original package at room temperature between +15 °C and +25 °C. In order to ensure the quality and shelf life of the BioSchiff reagent, keep BioSchiff reagent at 2-8°C after first opening. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Culling, C.F.A.(1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Davey, F.R. et Nelson, D.A.(1977): Periodic Acid Schiff (PAS) Stain. IN Hematology, 2nd ed., W. J. Williams, E. Buettler, A. J. Erslev, R.W. Rundles, McGraw-Hill, New York, p 1630-1632.
3. Hotchkiss, R.D.(1948): A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations, *Arch. Biochem.* 16, p 131.
4. Sheehan D.C. et Hrapchak, B.B.(1980): Theory an Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

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	European Conformity	°C	Storage temperature range		Number of tests in package		Product code
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile		Manufacturer

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

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