

LEUKOGNOST ALP

IVD *In vitro* diagnostic medical device



Kit for detection of alkaline phosphatase activity in leukocytes

INSTRUCTIONS FOR USE

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

Introduction

LeukoGnost ALP kit contains reagents for cytochemical diagnosis of leukemia using bone marrow or whole blood smears. The staining method is based on the ability of alkaline phosphatase of leukocytes to hydrolyze phosphate esters in alkaline solution. The reaction releases free naphthol that binds to diazonium salts to give a non-soluble brown precipitate at the reaction site.

The kit is intended for individual testing of horizontally placed slides and it contains reagents for at least 100 tests for detecting alkaline phosphatase activity in leukocytes. The reagents are applied by dripping until the entire slide is covered (1-2 mL).

Product description

- LEUKOGNOST ALP - kit for detection of alkaline phosphatase activity in leukocytes

The kit contains:	LKG-ALP (for 100 tests)	Storage temperature:
Reagent 1 (Sodium nitrite, solution)	NAN-OT-5 (5 mL)	2-8°C
Reagent 2 (Variamin Blue B, solution)	VAR-OT-3 (3 mL)	2-8°C
Reagent 3 (ALP buffer)	TRIS-OT-100 (2x100 mL)	2-8°C
Reagent 4 (ALP substrate)	NAF-OT-10 (2x10 mL)	2-8°C

Other reagents necessary for the staining method

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

or

- LeukoGnost PLUS (LKG-PLUS) – set of ancillary 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)**

Preparing the solution for staining

Prepare the staining solution in the following way:

- step 1: mix Reagent 1 and Reagent 2 in a clean tube. Let it set for 2 mins
- step 2: add Reagent 3 to mixture of Reagents 1 and 2
- step 3: add Reagent 4 to the prepared mixture of Reagents from step 2

Modify the reagents' volume as necessary:

STEP	REAGENT	FOR 1 SECTION	FOR 12 SECTIONS	FOR 24 SECTIONS
step 1	reagent 1	50 µL (1 drop)	600 µL (12 drops)	1.2 mL (24 drops)
	reagent 2	25 µL (1 drop)	300 µL (12 drops)	600 µL (24 drops)
step 2	reagent 3	2 mL	24 mL	48 mL
step 3	reagent 4	200 µL (4 drops)	2.4 mL	4.8 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 enzyme 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 in distilled 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.

Prepare fresh staining solution priori to each staining. The prepared solution must be used within 45 minutes.

Sample staining procedure

1.	Apply staining solution (1-2 mL) onto the slide	10 min
2.	Rinse the slide in distilled water thoroughly	10 seconds
3.	Dry the slide	
4.	Stain the slide using LeukoGnost HEM reagent	2 min
5.	Rinse the slide under tap water	2 min
6.	Dry the preparation	

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

Result

Cells in final stage of granulopoiesis - brown granulated cytoplasmic staining.

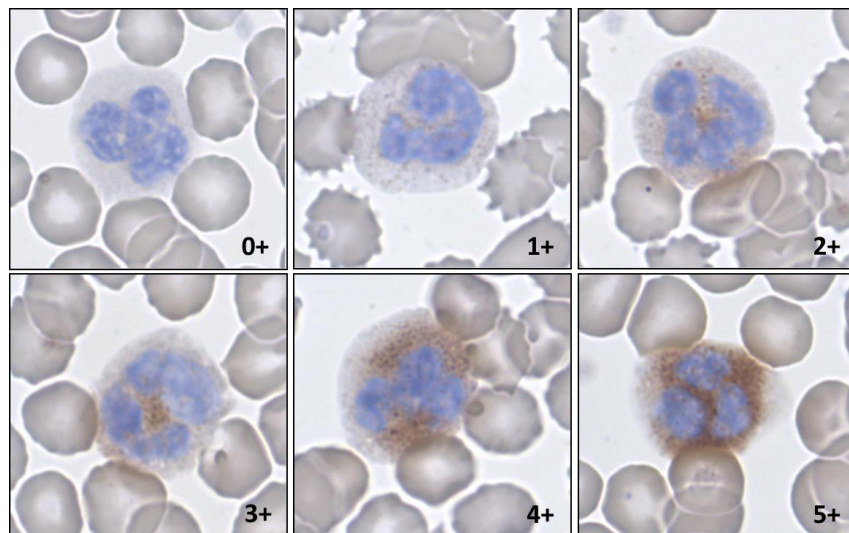


Figure 1. Blood smears stained with LeukoGnost ALP kit. Specifically stained neutrophils are shown. Magnification level is 1000x.

Staining gradation:

0+: no staining

1+: sporadic granules, very weak staining

2+: larger amount of granules that provide weak staining

3+: larger amount of granules that provide moderately strong staining

4+: large amount of granules that provide intensive staining

5+: exceptionally large amount of granules that provide almost homogeneous staining

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 ALP kit's reagents in a tightly closed original packaging at temperature between +2 °C and +8 °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. Carson, F.L. et Hladik, C. (2009): Histology, 3rd ed., American Society for Clinical Pathology Press, Hong Kong.
2. Lam KW, Li CY, Yam LT. Simultaneous demonstration of nonspecific esterase and chloroacetate esterase in human blood cells. Stain Technol. 1985;60:169-72.
3. Shibata A, Bennett JM, Castoldi GL, Catovsky D, Flandrin G, Jaffe ES, Katayama I, Nanba K, Schmalzl F, Yam LT, et al. Recommended methods for cytological procedures in haematology. International Committee for Standardization in Haematology (ICSH). Clin Lab Haematol. 1985;7:55-74.

LKG-ALP, V7-EN2, 19 August 2022, SB/IŠP

	European Conformity		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

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