BIOGNOST®

LEUKOGNOST ACP

IVD *In vitro* diagnostic medical device CE

Kit for detection of acid phosphatase activity in leukocytes

INSTRUCTIONS FOR USE

REF Product code: LKG-ACP (for at least 50 tests)

Introduction

LeukoGnost ACP kit contains reagents for cytochemical diagnosis of leukemia using bone marrow or whole blood smears. The method is based on the ability of the cellular phosphatase to hidrolyze naphthol AS-BI phosphate in acid environment. The reaction releases naphthol AS-BI that binds diazonium salts to give a red-brown precipitate at the reaction site.
The kit is intended for individual testing of horizontally placed slides and it contains reagents for at least 50 tests for detecting acid phosphatase activity in leukocytes. The reagents are applied by dripping until the entire slide is covered (1-2 mL).

Product description

- **LEUKOGNOST ACP** – kit for detection of the acid phosphatase activity in leukocytes

The kit contains:	LKG-ACP (for 50 tests)	Storage temperature:
Reagent 1 (Sodium nitrite, solution)	NAN-OT-5 (5 mL)	2-8°C
Reagent 2 (Fast Garnet GBC, solution)	FGGBC-OT-2 (2 mL)	2-8°C
Reagent 3 (ACP buffer)	NAP-OT-100 (100 mL)	2-8°C
Reagent 4 (ACP substrate)	NASBI-OT-5 (5 mL)	2-8°C
Reagent 5 (ACP inhibitor)	NATA-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 (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)**

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
- step 4 (option including acid phosphatase inhibition): add Reagent 5 to the mixture from step 3

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	100 µL (4 drops)	1.2 mL	2.4 mL
step 4 (optionally: with inhibition)	reagent 5	400 µL (8 drops)	4.8 mL	9.6 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 do +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.	Add the staining solution (with or without phosphatase inhibitor) (1-2 mL) onto the slide	3 hours
2.	Rinse the slide in distilled water thoroughly	10 seconds
3.	Dry the slide	
4.	Stain the slide using LeukoGnost HEM reagent	15 min
5.	Rinse the slide under tap water	3 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

Most leukocytes - granulated red staining in cytoplasm; there is no coloration in case of using the staining solution with the acid phosphatase inhibitor.

When using the staining solution that includes acid phosphatase inhibitor, there is no specific staining of most leukocytes; the granulated red staining of cytoplasm appears only in case of hairy cell leukemia.

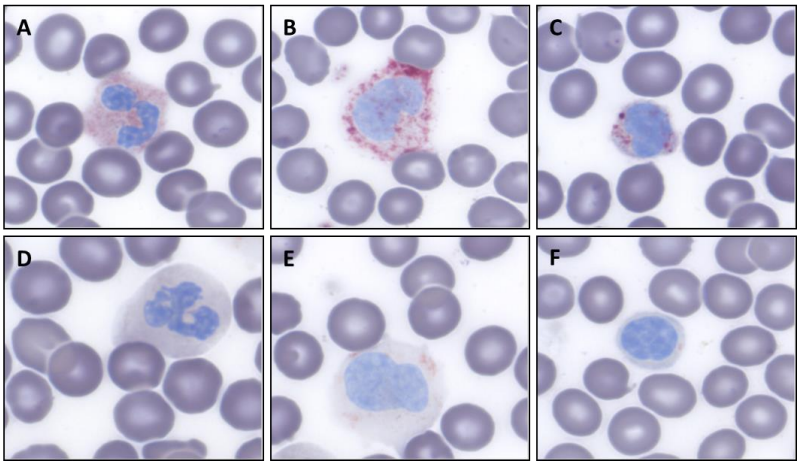

Figure 1. *Blood smears stained with LeukoGnost ACP kit. Neutrophils (A, D), monocytes (B, E), and lymphocytes (C, F) are shown. Staining conducted without (A, B, C) or with (D, E, F) acid phosphatase inhibitor. Magnification level is 1000x.*

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 ACP 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. Crook, L., Liu, P.I., Cannon, A., Walker Jr.E.M. Histochemistry of Bone Marrow Aspirations. Ann Clin Lab Sci. 1980;10:290-304.
3. Li, C.Y., Yam, L.T., Lam, K.W. Acid Phosphatase Isoenzyme in Human Leukocytes in Normal and Pathologic Conditions. J Histochem Cytochem. 1970;18:473-481.
4. Morse, E.E., Quinn, J., Altman, A., Talaizedeh, M., Brassel, J., Taubman, S. The Use of Leukocyte Acid Phosphatase in the Diagnosis of Malignant Disease. Case Report and Review of Literature. Ann Clin Lab Sci. 1980;10:143-8.
5. Shibata, A., Bennett, J.M., Castoldi, G.L., Catovsky, D., Flandrin, G., Jaffe, E.S., Katayama, I., Nanba, K., Schmalzl, F., Yam, L.T., et al. Recommended methods for cytological procedures in haematology. International Committee for Standardization in Haematology (ICSH). Clin Lab Haematol. 1985;7:55-74.

LKG-ACP, V7-EN2, 18 August 2022, SB/IŠP

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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