BIOGNOST®

LEUKOGNOST NAP

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 – **Class A** product

Kit for detection of alkaline phosphathase activity in neutrophil leukocytes

INSTRUCTIONS FOR USE

Basic UDI number	385889212HPC30702CYTOS9	
EMDN code	W01030702	
REF **Catalogue number**	**Volume**	**UDI-DI number**
LKG-NAP	For at least 100 tests	03858892126240

Introduction

The LeukoGnost NAP Kit contains reagents for the cytochemical diagnosis of leukemias on peripheral blood or bone marrow smears. This kit enables differentiation of chronic myeloid leukemia (CML) from a leukemoid reaction and other myeloproliferative disorders. The method is based on the ability of alkaline phosphatase in neutrophil leukocytes to catalyze the hydrolysis of naphthol AS-BI phosphate in an alkaline solution. This reaction produces free naphthol AS-BI, which reacts with diazo salts to form an insoluble blue precipitate at the site of the reaction. The kit is designed for individual testing of horizontally positioned slides and contains reagents sufficient for at least 100 tests for the detection of alkaline phosphatase activity in leukocytes. The reagents are applied dropwise until the entire slide is covered (1–2 mL).

Product description

- **LeukoGnost NAP** – Kit for detection of alkaline phosphathase activity in neutrophil leukocytes

The kit contains:	LKG-NAP (for 100 tests)	Storage temperature:
Reagent 1 (Sodium nitrite, solution)	NAN-OT-5 (5 mL)	2-8°C
Reagent 2 (Fast Blue BB, solution)	FBBB-OT-10 (10 mL)	2-8°C
Reagent 3 (NAP buffer)	TRIS-OT-100 (2x100 mL)	2-8°C
Reagent 4 (NAP substrate)	NAPI-OT-10 (10 mL)	2-8°C
Reagent 5 (Neutral Red, solution)	NRED-OT-100 mL (2x100 mL)	2-8°C

Other reagents necessary for the staining method

- **LeukoGnost Fiksativ (LKF-500)** – fixative for use in cytochemical diagnosis of leukemia

Other reagents that may be used with the staining procedure

- 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 the 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	100 µL (2 drops)	1,2 mL (24 drops)	2,4 mL (48 drops)
step 2	Reagent 3	2 mL	24 mL	48 mL
step 3	Reagent 4	100 µL (2 drops)	1,2 mL (24 drops)	2,4 mL (48 drops)

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 whitin 3 days

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.

Incubate the samples for 15 minutes at room temperature (15–25 °C) to avoid changes in enzymatic activity. Perform the test using positive and negative controls. Positive controls may include blood samples from patients with infectious leukocytosis, pregnant women in the third trimester, and healthy individuals. A negative control can be obtained by immersing a fixed slide in boiling water for 1 minute.

The enzyme is present almost exclusively in the cytoplasm of mature neutrophil leukocytes. In non-segmented neutrophil leukocytes, the activity is less pronounced. A significantly positive reaction is also observed in reticuloendothelial cells on bone marrow smears.

Staining procedure

1.	Apply the staining solution (1-2 mL) to the slide and protect it from direct sunlight	15 minutes
2.	Rinse the slide thoroughly with distilled/demineralized water and do not allow it to dry	10 seconds
3.	Stain the slide with Neutral Red reagent	2 minutes
4.	Rinse the slide with distilled/demineralized water	2 minutes
5.	Allow the slide to air dry	

Note: Neutral Red is a stain that acts as a pH indicator and changes color from red to yellow in the pH range of 6.8–8.0. To preserve the red color of leukocyte nuclei, use rinse water with a pH below 6.8. If fading of leukocyte nuclei is observed during slide evaluation, check the pH of the water. If a coverslip needs to be mounted, use an aqueous-based mounting medium.

Result

Cells in the final stage of granulopoiesis – blue granular staining of the cytoplasm

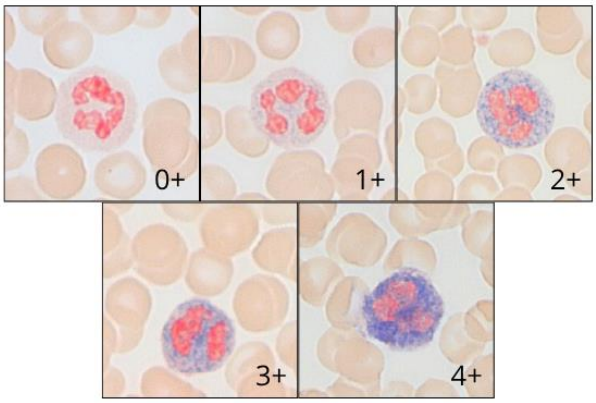

Figure 1. *Illustration of a normal blood smear stained with the LeukoGnost NAP Kit. Specifically stained neutrophil leukocytes are shown.*

Staining intensity grading in five categories, modified according to Kaplow:
0+: no staining
1+: sporadic granules, very weak staining
2+: a greater number of granules producing weak staining
3+: a greater number of granules producing moderately strong staining
4+: a large number of intensely stained granules producing strong staining

Examine the slide in the thin area where erythrocytes do not overlap (at 1000× magnification). Evaluate the staining intensity of 100 consecutive neutrophil leukocytes according to Figure 1. Multiply the number of neutrophils by the corresponding staining grade. The sum of these values represents the overall staining score of the slide (NAP score), which in healthy individuals ranges from 13 to 130.
The active phase of chronic myeloid leukemia is characterized by a significantly reduced NAP score, whereas in a leukemoid reaction the score is elevated. It is recommended that each laboratory establish its own reference interval based on its patient population.

Limitations

This product is intended for professional laboratory use only for diagnostic purposes. Any deviation from the staining procedure described in these Instructions for Use may result in variations in staining results.

Sample preparation and diagnostics

Use appropriate instruments for sample collection and preparation. Process samples using current technologies and ensure they are clearly labeled. Always follow the manufacturer's handling instructions. To avoid errors, sample staining and diagnostic evaluation must be performed only by qualified personnel. Use a microscope that meets the standards of a medical diagnostic laboratory. To minimize the risk of incorrect results, the use of positive and negative controls is recommended. If a serious incident occurs during the use of this product or as a result of its use, please report it to the manufacturer and/or the authorized representative, as well as to the competent authority.

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

Upon receipt, store the product in a dry place, in the tightly closed original packaging, at a temperature of +2 °C to +8 °C. Do not freeze and do not expose to direct sunlight. After first opening, the product may be used until the stated expiration date, provided it is stored correctly. The date of manufacture and expiry date are printed on the product label.

References

1. Carson, F.L. et Hladik, C. (2009): Histology, 3 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.
4. Kaplow LS. A histochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood. 1955;10(10):1023-9.
5. Kaplow LS. Cytochemistry of leukocyte alkaline phosphatase. Use of complex naphthol as phosphates in azo dyecoupling technics. Am J Clin Pathol. 1963;39:439-49.

Warnings and precautions regarding the materials contained in the product:

H290	May be corrosive to metals.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
P234	Keep only in original container/packaging.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water (or shower).
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

LKG-NAP-IFU, ENV1, 16.1.2026., IŠP

	Manufacturer	LOT	Batch code		Consult Instructions for use	Σ	Contains sufficient for <n>tests
	Date of manufacture	REF	Catalogue number		Caution	CE	European conformity
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device	UDI	Unique device identifier

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Version	Description / reason for change	Date
1.	First version of the Instruction for use	16.01.2026.