

# LEUKOGNOST NSE

IVD *In vitro* diagnostic medical device



## Kit for detection of non-specific esterase activity in leukocytes

### INSTRUCTIONS FOR USE

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

#### Introduction

LeukoGnost NSE kit contains reagents for cytochemical diagnosis of leukemia using bone marrow or whole blood smears. The staining method is based on the ability of cellular esterase to hydrolyze 1-naphthyl acetate. The reaction releases free naphthol that binds to 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 100 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 NSE** - kit for detection of non-specific esterase activity in leukocytes

The kit contains:	LKG-NSE (for 100 tests)	Storage temperature:
Reagent 1 (Sodium nitrite, solution)	NAN-OT-5 (5 mL)	2-8°C
Reagent 2 (Pararosaniline, solution)	PARA-OT-3 (3 mL)	2-8°C
Reagent 3 (NSE buffer)	NSEP-OT-100 (2x100 mL)	2-8°C
Reagent 4 (NSE substrate)	NAFA-OT-10 (10 mL)	2-8°C
Reagent 5 (NSE inhibitor)	NAFO-OT-15 (3x15 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 non-specific esterase 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 sample 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

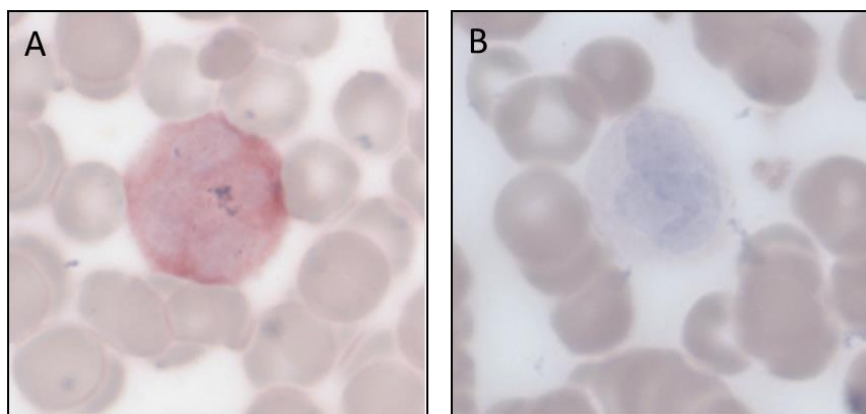
1.	Apply the staining solution (with or without esterase inhibitor) (1-2 mL) onto the slide	1 hour 30 mins
2.	Rinse the slide in distilled water vigorously	10 seconds
3.	Stain the slide using LeukoGnost HEM reagent	15 min
4.	Rinse the slide under tap water	2 min
5.	Dry the preparation	

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

Monocytes, plasma cells, erythroblasts, megakaryocytes, monocyte leukemia cells - brown-red granular cytoplasmic staining

When using the staining solution that includes non-specific esterase inhibitor, there is no specific staining of monocytes, plasma cells, erythroblasts, megakaryocytes and monocytic leukemia cells.



**Figure 1.** Blood smears stained with LeukoGnost NSE kit. The smears are stained without (A) or with (B) non-specific esterase inhibitor. Monocytes are shown. 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 NSE 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, 3<sup>rd</sup> 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-NSE, V9-EN2, 18 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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