BIOGNOST®

LEUKOGNOST SPENSE

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

Kit for simultaneous detection of specific and non-specific esterase activity in leukocytes

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30702CYTOS9	
EMDN code	W01030702	
REF **Catalog number**	**Volume**	**UDI-DI**
LKG-SPENSE	for at least 50 tests	03858892123409

Intended use and test principle

This kit contains reagents for the cytochemical diagnosis of leukemias on whole blood or bone marrow smears. The method is based on the ability of one type of cellular esterase to hydrolyze naphthol AS-D chloroacetate and the ability of another type of cellular esterase to hydrolyze 1-naphthyl acetate. In both types of reaction, free naphthols are formed which, with different diazo salts, create insoluble precipitates at the site of the reaction. The kit is designed for individual testing of horizontally positioned slides and contains reagents for at least 50 tests for detecting specific and non-specific esterase activity in leukocytes. The reagents are applied by dripping until the entire slide is covered (1-2 mL).

Product description

- **LeukoGnost SPENSE -** kit for simultaneous detection of specific and non-specific esterase in leukocytes

The kit contains:	LKG-SPENSE (for 50 tests)	Storage temperature:
Reagent 1 (Sodium nitrite, solution)	NAN-OT-5 (5 mL)	2-8°C
Reagent 2 (Fast Blue RR/Fast Garnet GBC)	FBFG-OT-2 (2 mL)	2-8°C
Reagent 3 (SPE buffer)	FOP-OT-100 (100 mL)	2-8°C
Reagent 4 (SPE substrate)	NASD-OT-5 (5 mL)	2-8°C
Reagent 5 (Fast Garnet GBC, solution)	FGGBC-OT-2 (2 mL)	2-8°C
Reagent 6 (NSE buffer)	NSEP-OT-100 (100 mL)	2-8°C
Reagent 7 (NSE substrate)	NAFA-OT-5 (5 mL)	2-8°C
Reagent 8 (NSE inhibitor)	NAFO-OT-10 (2x10 mL)	2-8°C
Reagent 9 (Methyl Green, solution)	MGR-OT-100 (100 mL)	2-8°C

Other reagents required in the staining method

- **LeukoGnost Fixative (LKF-500)** – Fixative for use in the cytochemical diagnosis of leukemias
- **LeukoGnost HEM (LKH-OT-500)** – Hematoxylin for use in the cytochemical diagnosis of leukemias

or

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

Other preparations and reagents that may be used in the staining method

- Water-based agent for covering microscopic samples and mounting cover glasses such as BioGnost's BioMount Aqua (BMA-30)
- BioGnost's immersion media such as Immersion Oil (IU-30) or Immersion Oil Type A (IUA-30)

Preparation of staining samples:

- Prepare the peripheral blood or bone marrow smear to be thin and dry (dry for at least 30 minutes). Samples must not be older than three days.
- It is not recommended to use anticoagulants as inhibition of the reaction may occur.
- Fix the sample as follows:

1.	Fix the sample by applying LeukoGnost Fixative (1-2 mL) to the slide	1-3 minutes
2.	Rinse the slide under running tap water	10 seconds
3.	Air dry the slide	

- Slides prepared and fixed in this way can be stored at 2-8 °C and used for up to three days.

NOTE

Apply the reagent so that it completely covers the slide.

Prepare the staining solutions fresh before each staining (the solution slide procedure is described below the staining procedure table). The prepared solutions can be used within 30 minutes.

Sample staining procedure

1.	Apply staining solution A (1-2 mL) to the slide	30 minutes
2.	Rinse vigorously in distilled/demineralized water	10 seconds
3.	Apply staining solution B (with and/or without esterase inhibitor) (1-2 mL) to the slide	90 minutes
4.	Rinse vigorously in distilled/demineralized water	10 seconds
5.	Stain the slide with Methyl Green solution	5 minutes
6.	Briefly rinse the slide with water	
7.	Air dry the slide	

After drying the slide, it is recommended to mount a cover glass using BioMount Aqua to preserve the stain and quality of the slide.

PREPARATION OF STAINING SOLUTIONS

1. Prepare staining solution A as follows:

- step 1: mix Reagent 1 and Reagent 2 in a clean test tube. Allow to stand for 2 min.
- step 2: add Reagent 3 to the mixture of Reagent 1 and 2.
- step 3: add Reagent 4 to the prepared mixture from step 2.

Adjust the reagent volume as needed:

STEP	REAGENT	FOR 1 SLIDE	FOR 12 SLIDES	FOR 24 SLIDES
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

2. Prepare staining solution B as follows:

- step 1: mix Reagent 1 and Reagent 5 in a clean test tube. Allow to stand for 2 min.
- step 2: add Reagent 6 to the mixture of Reagent 1 and 5.
- step 3: add Reagent 7 to the prepared mixture from step 2.
- step 4 (option with inhibition of non-specific esterase): add Reagent 8 to the mixture from step 3.

Adjust the reagent volume as needed:

STEP	REAGENT	FOR 1 SLIDE	FOR 12 SLIDES	FOR 24 SLIDES

step 1	Reagent 1	50 µL(1 drop)	600 µL(12 drops)	1.2 mL (24 drops)
	Reagent 5	25 µL (1 drop)	300 µL (12 drops)	600 µL (24 drops)
step 2	Reagent 6	2 mL	24 mL	48 mL
step 3	Reagent 7	100 µL(4 drops)	1.2 mL	2.4 mL
step 4 (optional: with inhibition)	Reagent 8	400 µL(8 drops)	4.8 mL	9.6 mL

Result

Neutrophils, promyelocytes and myeloblastic leukemia cells – blue to blue-violet cytoplasmic staining, faintly green stained nuclei.
Monocytes – brown to brownish-red cytoplasmic staining, faintly green stained nuclei. When using staining solution B that includes a non-specific esterase inhibitor, no specific staining occurs.

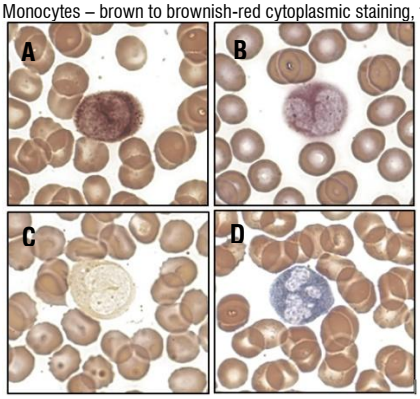

Figure 1. *Display of peripheral blood smears stained with the LeukoGnost SPENSE kit. Specifically stained monocytes (A-brown staining, B-red staining) and a neutrophil (D) are shown, as well as an unstained monocyte with a non-specific esterase inhibitor (C). Magnification 1000x.*

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instructions for Use may cause differences in staining results.

Sample slide and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining of samples and making a diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control.
If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the notice and warning signs printed on the product label and in the BioGnost Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store and keep the product in a dry place, in the original tightly closed packaging at a temperature of +2 to +8°C. The product is transported at a temperature of +2 to +8°C. Do not freeze and do not expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration 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.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapour.
H302	Harmful if swallowed.
H312	Harmful in contact with skin.
H319	Causes severe eye irritation.
H317	May cause an allergic skin reaction.
H332	Harmful if inhaled.
H350	May cause cancer.
H370	Causes damage to organs.
H360D	May damage the unborn child.
P201	Obtain special instructions before use.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. Do not smoke.P234
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P261	Avoid breathing dust/fume/gas/mist/vapors/spray.
P264	Wash affected parts thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.
P302+P352	IF ON SKIN: wash with plenty of water.
P303+P361+P353	IF ON SKIN (or hair): immediately take off all contaminated clothing. Rinse skin with water [or shower].
P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
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-SPENSE-IFU, ENV4, 29.07.2026., IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	Σ	Contains sufficient for <n>tests		Contains hazardous substances
	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		

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

Version	Description / reason for change	Date
4	Revised acc. to Regulation (EU) 2017/746 - IVDR	29.07.2026.