BIOGNOST®

LEUKOGNOST SPE

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

Kit for detection of specific esterase activity (Leder stain, chloroacetate esterase) in leukocytes

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30702CYTOS9	
EMDN code	W01030702	
REF **Catalog number**	**Volume**	**UDI-DI**
LKG-SPE	for at least 100 tests	03858892122457

Intended use and test principle

The LeukoGnost SPE kit contains reagents for cytochemical diagnosis of leukemia on peripheral blood or bone marrow smears. This staining method can be used on blood smears as well as bone marrow smears, and also on histological bone marrow preparations. The method is based on the ability of cellular esterase to hydrolyze naphthol AS-D chloroacetate. In this reaction, free naphthol is produced, which reacts with diazonium salts to form an insoluble red precipitate at the site of the reaction. The kit is designed for individual testing of horizontally placed slides and contains reagents for at least 100 tests for the detection of specific esterase activity in leukocytes. The reagents are applied by dripping until the entire preparation is covered (1-2 mL).

Product description

- **LEUKOGNOST SPE** – kit for detection of specific esterase activity in leukocytes

The kit contains:	**LKG-SPE (for 100 tests)**	**Storage temperature**
Reagent 1 (Sodium nitrite, solution)	NAN-OT-5 (5 mL)	2-8°C
Reagent 2 (Pararosanilin, solution)	PARA-OT-3 (3 mL)	2-8°C
Reagent 3 (SPE buffer)	FOP-OT-100 (2x100 mL)	2-8°C
Reagent 4 (SPE substrate)	NASD-OT-10 (10 mL)	2-8°C

Other reagents required in 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 additional reagents for LeukoGnost kits

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

- Water-based agent for covering microscopic preparations 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)

Prepare the staining solution 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 PREPARATION**	**FOR 12 PREPARATIONS**	**FOR 24 PREPARATIONS**
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

Preparation of samples for staining

- 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 preparation 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 solution fresh before each staining. The prepared solution can be used within 45 minutes.

Sample staining procedure

A) Staining procedure for blood smears and bone marrow smears

1.	Apply the staining solution (1-2 mL) to the preparation	30 minutes
2.	Rinse the preparation vigorously in distilled water	10 seconds
3.	Stain the preparation with LeukoGnost HEM reagent	5 minutes
4.	Rinse the preparation under running tap water	5 minutes
5.	Air dry the slide	

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

Preparation of histological samples for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water, and dehydrate through a series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95, and Histanol 100).
- Clear the sample with an intermediary agent; xylene (BioClear) or xylene substitute (BioClear New).
- Infiltrate and embed the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block into 4-6 micron thin sections and mount on a VitroGnost microscope slide.

B) Staining procedure for histological preparations

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 minutes each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 minutes
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydrate in distilled/demineralized water	2 minutes
5.	Apply the staining solution to the slide (2 mL)	30 minutes

6.	Carefully rinse in distilled/demineralized water	10 seconds
7.	Stain the slide with LeukoGnost HEM reagent	5 minutes
8.	Rinse the slide under running tap water	5 minutes
9.	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.

Result

Neutrophils, promyelocytes, and cells of myeloblastic leukemia – intense red granular staining of the cytoplasm.
Monocytes – rarely show weak red granular staining of the cytoplasm.

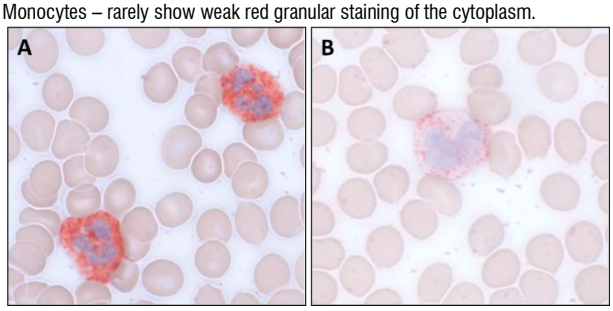

Figure 1. *Peripheral blood smear stained with the LeukoGnost SPE kit. Specifically stained neutrophils (A) and a monocyte (B) are shown. Magnification 1000x.*

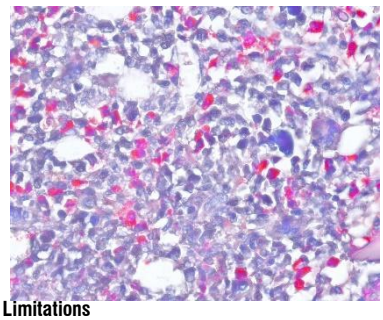

Figure 2*. Histological bone marrow preparation stained with the LeukoGnost SPE kit. Positive reaction of myeloid lineage cells in acute myeloid leukemia (AML) is shown.Magnification 200x.*

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 preparation 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, the staining procedure and diagnosis may only be performed by authorized and professionally trained personnel. Use a microscope that complies with medical diagnostic laboratory standards.

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 following 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 information 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:

H302	Harmful if swallowed.
H312	Harmful in contact with skin.
H332	Harmful if inhaled.
H350	May cause cancer.
H319	Causes severe eye irritation.
H360D	May damage the unborn child.
P201	Obtain special instructions before use.
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.
P251	Do not pierce or burn, even after use.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P302+P352	IF ON SKIN: wash with plenty of water
P305+P351+P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
P332+P337+P313	If skin irritation occurs: If eye irritation persists: get medical advice/attention.

LKG-SPE-IFU, ENV3, 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		

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