LEUKOGNOST SPE

IVD *In vitro* diagnostic medical device CE

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

INSTRUCTIONS FOR USE

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

Introduction

LeukoGnost SPE kit contains reagents for cytochemical diagnosis of leukemia using bone marrow or whole blood smears. This method can be also used on formalin fixed and paraffin embedded material, especially on histological preparations of bone marrow.
The staining method is based on the ability of the cellular esterase to hydrolyze naphthol AS-D chloroacetate. The reaction releases free naphthol that binds to diazonium salts to give a red 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 SPE -** kit for detection of specific esterase activity (Leder stain, chloroacetate esterase) 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 (Pararosaniline, 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 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)**
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.

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

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

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 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.

A) Blood smears and bone marrow smears staining procedure

1.	Apply staining solution (1-2 mL) onto the slide	30 min
2.	Rinse the slide in distilled water thoroughly	10 seconds
3.	Stain the slide using LeukoGnost HEM reagent	5 min

4.	Rinse the slide under tap water	5 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.

Preparing the histology sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

B) Histology sections staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Apply the staining solution on the section (2 mL)	30 min
6.	Carefully rinse in distilled water	10 sec
7.	Stain the section with LeukoGnost HEM reagent	5 min
8.	Rinse the slide under tap water	5 min
9.	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

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

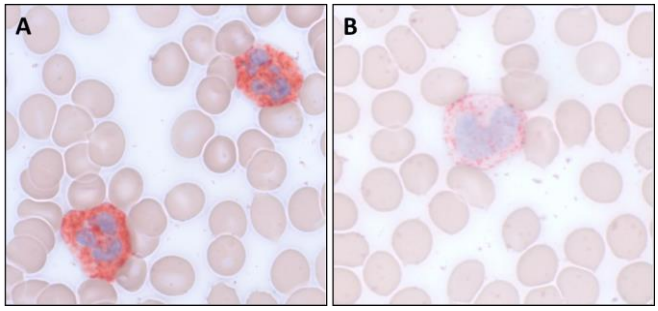

Figure 1. *Blood smears stained with LeukoGnost SPE kit. Specific staining of neutrophils (A) and monocyte (B) are shown.*

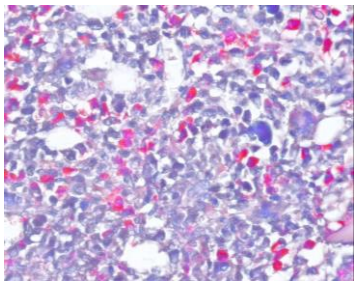

Figure 2. *Bone marrow (histological slide) stained with LeukoGnost SPE kit. Positive reaction with cells of the myeloic cell line in acute myeloid leukemia (AML) is shown. Magnification level is 200x.*

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 SPE 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. 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-SPE, V8-EN2, 26 September 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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