

LEUKOGNOST SPENSE

IVD *In vitro* diagnostic medical device



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

INSTRUCTIONS FOR USE

REF Product code: LKG-SPENSE (for at least 50 tests)

Introduction

This kit contains reagents for cytochemical diagnosis of leukemia using bone marrow or whole blood smears. The method is based on the capability of one type of cell esterase to hydrolyze naphthol AS-D chloroacetate and the capability of another type of cell esterase to hydrolyze 1-naphthyl acetate. Both reactions create free naphthols that bind to various diazonium salts to give a non-soluble precipitates at the reaction site. The kit is intended for individual testing of horizontally placed sections and it 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 section is covered (1-2 mL).

Product description

- LEUKOGNOST SPENSE - kit for simultaneous detection of specific and non-specific esterase activity 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, solution)	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 necessary for the staining method

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

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 (BMA-30)**
- BioGnost's immersion oils, such as **Immersion oil (IU-30)** or **Immersion oil type A (IUA-30)**

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 solutions priori to each staining. The prepared solutions must be used within 30 minutes.

Sample staining procedure

1.	Apply staining solution (1-2 mL) onto the slide	30 minutes
2.	Rinse the slide in distilled water vigorously	10 seconds
3.	Add the staining solution B (with or without esterase inhibitor) (1-2 mL) onto the slide	90 minutes
4.	Rinse the slide in distilled water vigorously	10 seconds
5.	Stain the slide with Methyl Green, solution	5 minutes
6.	Rinse the slide shortly using tap water	
7.	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 SOLUTIONS FOR STAINING

1. Prepare staining solution A 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

2. Prepare the staining solution B in the following way:

- step 1: mix Reagent 1 and Reagent 5 in a clean tube. Let it set for 2 mins.
- step 2: add Reagent 6 to mixture of Reagents 1 and 5
- step 3: add Reagent 7 to the prepared mixture of Reagents from step 2
- step 4 (option including non-specific esterase inhibition): add Reagent 8 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 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 (optionally: with inhibition)	reagent 8	400 µL (8 drops)	4.8 mL	9.6 mL

Result

Neutrophils, promyelocytes and myeloblastic leukemia cells - blue to bluish-purple staining of the cytoplasm, slightly green-stained nuclei.
Monocytes - brown to brown-red cytoplasmic staining, slightly green stained nuclei. When using staining solution B that includes a non-specific esterase inhibitor, no specific staining occurs.

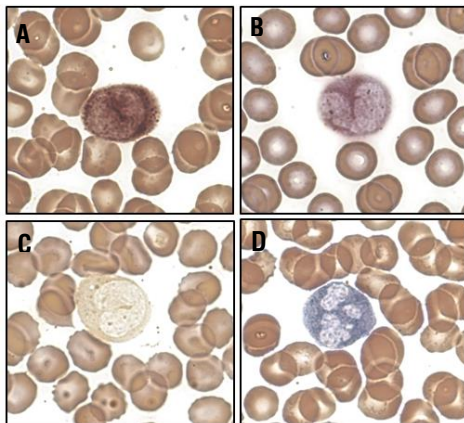


Figure 1 Blood smears stained with LeukoGnost SPENSE kit. Specifically stained monocytes (A-brown staining, B-red staining) and neutrophils (D) and unstained monocyte with non-specific esterase inhibitor (C) are shown. Magnification level is 1000x.

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 SPENSE 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 Hladiik, 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.
4. Yam LT, Li CY, Crosby WH. Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol. 1971;55:283-90.

LKG-SPENSE, V7-EN3, 19 August 2022, SB/ISP

	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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