

SUDAN BLACK B ECO KIT

IVD In vitro diagnostic medical device

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Three-reagent kit for staining neutrophil granules in hematology smears **INSTRUCTIONS FOR USE**

REF Product code: SBBE-100T (for at least 100 tests)

Introduction

BioGnost's Sudan Black B Eco kit is a phenol-free product making this kit more reliable and safer compared to other kits of the same type and purpose. It is used in cytochemistry for staining neutrophil granules in blood smears and bone marrow smears. The result of staining the leukocytes with Sudan Black B dye is similar to the result of staining with myeloperoxidase in leukocytes (for instance, LeukoGnost MPO). The difference compared to myeloperoxidase activity detection in leukocytes is in intensity of eosinophilic staining; they are more weakly stained using Sudan Black B kit. Cells of lymphoid line will not be stained with Sudan Black B dye, while myeloid and monocytoid cells will demonstrate characteristic positive reaction. This is the reason why Sudan Black B is used in methods of determining myelocytic and myelomonocytic leukemia.

Kit is designed for individual testing of horizontally placed sections and contains reagents sufficient for at least 100 tests of specific esterase activity detection in leukocytes. Reagents are applied until the section is entirely covered.

Product description

SUDAN BLACK B ECO KIT - Kit for staining neutrophil granules

The kit contains:	SBBE-100T (for 100 tests)	Storage temperature:
Sudan Black B fixative	SBBF-100 (100 ml)	15-25°C
Sudan Black B reagent, phenol free	SBBP-0T-100 (100 ml)	15-25°C
LeukoGnost HEM	LKH-0T-100 (100 ml)	15-25°C

Other sections and reagents that may be used with the staining procedure

- Aqueous covering medium for microscope sections and mounting medium for 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)
- 70% ethanol, such as BioGnost's Histanol 70 (H70-1L)

Hematological smears staining procedure

Preparing the section for staining

- . Whole blood or bone marrow sample should be thin and dry. Such sections must not be older than three days. It is not recommended to use anticoagulants because they can cause enzyme reaction inhibition.
- Fix the section using the following method:

Γ	1.	Fix the section using Sudan Black B fixative	30 seconds
Γ	2.	Rinse the section in distilled water	10 seconds
Γ	3.	Dry the preparation	

Sections fixed using this method may be stored at temperature between 2 and 8 °C and used for three days at most.

Apply the reagent so it completely covers the section. If necessary, filter Sudan Black B reagent, phenol free.

Sample staining procedure

1.	Apply Sudan Black B reagent, phenol free (1 mL) on the section	3-5 minutes
2.	Rinse the section shortly in 70% ethanol	3 short dips
3.	Rinse the section with distilled water	20 seconds
4.	Stain the section using LeukoGnost HEM reagent	3 min
5.	Rinse the section under tap water.	2 min
6.	Dry the preparation	

After the section is dried, it is recommended to mount the cover slide using BioMount Aqua medium to preserve color and section quality.

Result

Neutrophil granules and neutrophil precursors - black Monocytes and eosinophiles - more weakly stained black granule Lymphocytes - not stained

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 Sudan Black B Eco kit in a tightly closed original packaging at temperature of $+15-25^{\circ}$ 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. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
- 2. Davey, F.R. et Nelson, D.A. (1977): Sudan Black B staining. IN Hematology, 2nd ed., W. J. Williams, E. Beutler, A. J. Erslev, R. W. Rundles, Editors, McGraw-Hill, New York, str. 1629-1630
- 3. Sheehan H.L. et Storey G.W. (1947): An improved method of staining leukocyte granules with Sudan Black, J. Pathol Bacteriol, 59:336

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