BIOGNOST®

SUDAN BLACK B KIT

IVD In vitro diagnostic medical device CE

Four-reagent kit for staining neutrophil granules in hematological smears

INSTRUCTIONS FOR USE

REF Catalog number: SBBH-K-100

Introduction

BioGnost's Sudan Black B kit for hematological staining in cytochemistry is used 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 result after staining with myeloperoxidase. 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.

Product description

- **SUDAN BLACK B KIT** - Kit for staining neutrophil granules

The kit contains:	SBBH-K-100
Sudan Black B reagent	2 x 100 mL (SBB-OT-100)
Hematoxylin G3	1 x 100 mL (HEMG3-OT-100)
Glutaraldehyde, 4% buffered solution	1 x 75 mL (G4-OT-75)
Acetone for histology	1 x 25 mL (AC-25)

Other sections, reagents and accessories that are used in the staining method:

- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparation of solutions

Glutaraldehyde fixative

- Pour all the contents of Acetone for histology (25 mL) into a bottle containing 75 mL of Glutaraldehyde, 4% buffered solution and mix well. The solution is stable for several months when stored in tightly closed packaging.

Hematological smears staining procedure

Notes:
- use only freshly sampled whole blood or anticoagulated blood, and bone marrow smear
- fix the samples in the shortest time period possible
- cool down glutaraldehyde fixation solution in refrigerator at 2-8°C before use
- if a precipitate appears in the reagent *Glutaraldehyde, 4% buffered solution*, it must be filtered (the precipitate does not affect the staining quality)

1.	**Cool glutaraldehyde fixative in refrigerator (2-8°C)**	
2.	Fix the smear by dipping it into glutaraldehyde fixative at 2-8°C with gentle agitation	1 min
3.	Thoroughly rinse in distilled (demi) water	
4.	Dip into Sudan Black B reagent with intermittent agitation	5 min
5.	Rinse 3 or more times in 70% alcohol (Histanol 70) until no more dye washes out	
6.	Thoroughly rinse in distilled (demi) water	
7.	Immerse in Hematoxylin G3	5 min
8.	Blue using Scott's solution or Bluing reagent, dip into the reagent	1 min
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
9.	Dry the sections	

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Result

Neutrophil granules and neutrophil precursors - blue-black
Monocytes - blue-black (lightly stained)
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 kit in a tightly closed original packaging at temperature of 15-25ºC. 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, pp 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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	Refer to the supplied documentation		Storage temperature range		Number of tests in package	REF	Product code	CE	European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile