BIOGNOST®

SUDAN III, C.I. 26100

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

For lipid staining acc. to Lillie-Ashburn

Sudan Red III, Solvent Red 23, Sudan G, Fat Ponceau G

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30707PDYETD	
EMDN code	W01030707	
REF **Catalog number**	**Mass**	**UDI-DI**
S3-P-25	25 g	03858888821340

Intended use and test principle

Histology, cytology, and other related scientific disciplines study the microscopic anatomy of tissues and cells. Proper staining is required to achieve good visualization of tissue and cellular structures. The principle of Sudan III staining is based on the solubility of the dye in lipids, rather than on chemical binding to tissue. Sudan III is a lipophilic (fat-soluble) dye that dissolves in droplets of neutral fats and concentrates there. As a result, lipid structures are stained orange-red to red. The method is most commonly applied to frozen sections, as lipids are largely removed from tissue during paraffin processing.

Product description

- **SUDAN III, C.I. 26100** - powder dye for the preparation of a staining solution for the staining of lipids and hydrophobic materials

Example of using Sudan III powder dye for staining lipids using the Lillie-Ashburn method

Additional reagents and materials that can be used in the method

- Fixatives, such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agent such as BioGnost's alcohol solution: Histanol 70
- Reagents for staining nuclei such as BioGnost Hematoxylin solutions (H, ML, G1, G2, G3 and Hematoxylin M)
- Nuclei bluing reagents such as BioGnost's Bluing reagent or Scott's solution
- VitroGnost slides and coverslips for use in histopathology and cytology
- Water-based agent for covering microscopic samples and mounting cover glasses such as BioGnost's BioMount Aqua (BMA-30)
- BioGnost's isopropyl alcohol Histanol IP (cat. no. HIP-1L)

Preparation of Sudan III staining solution

- Saturated Sudan III dye solution (100 mL):
  Dissolve 0.5 g of Sudan III powder dye in 100 mL of isopropyl alcohol (Histanol IP).Allow to stand for 2–3 days at room temperature, filter before use.
- Sudan III dye working solution:
  Dilute 6 mL of the saturated Sudan III dye solution with 4 mL of distilled/demineralized water. Allow to stand for 5–10 minutes, then filter the solution.
  Note: The filtrate can be used for several hours.

Preparation of the sample for staining

- Use a fresh sample or an unprocessed sample fixed in formalin
  Freezing procedure for unprocessed formalin-fixed tissue; rinse the sample under running water for 5 minutes, then dry thoroughly. Then freeze the sample according to the prescribed laboratory procedure.
- Cut the frozen sample into 8–10 micron thin sections and mount on an adhesive slide

NOTE

Apply the reagent so that it completely covers the section.

Staining procedure for frozen histological sections

1.	Fix the frozen section in 4% Formaldehyde NB	1 min
	Note: If frozen samples previously fixed in formalin are used, this step can be skipped	
2.	Carefully rinse through two changes of distilled/demineralized water	
3.	Rinse in 70% alcohol solution (Histanol 70)	
4.	Stain with Sudan III dye working solution	10 min
	Note: use a staining jar with a lid to prevent evaporation	
4.	Differentiate in 70% alcohol solution (Histanol 70) to remove excess dye	
5.	Rinse thoroughly in distilled/demineralized water	
6.	Counterstain with Hematoxylin ML	2–3 min
	Note: Hematoxylin H, Hematoxylin G2, or Hematoxylin G3 may also be used with the same incubation time	
7.	Carefully rinse through several changes of tap water	
8.	Make nuclei turn blue using Scott's solution or Bluing reagent	10 dips
9.	Note: Stop bluing after the nuclei turn blue If Scott's solution or Bluing reagent are unavailable, rinse the slides under running tap water for 3–5 minutes	
10.	Rinse in distilled/demineralized water	
11.	Carefully rinse through several changes of tap water	
12.	Remove excess water from the section	

Apply BioMount Aqua mounting medium. Cover the section with a VitroGnost cover glass. Note: Carefully mount the coverslip onto the stained sample with as little pressure as possible so as not to disturb the results of the stained fats, i.e., lipids. To remove air

bubbles under the coverslip, immerse the section in warm water and keep it submerged until the coverslip can be easily removed. Remove excess water from the section and re-mount with a new coverslip and water-based medium (BioMount Aqua).

Result

Lipids - orange-red
Nuclei - blue

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations in the preparation of solutions and/or from the staining procedure described in BioGnost's 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 preparation of staining solutions, the staining procedure, and the establishment of a diagnosis may only be performed by qualified personnel.
Use a microscope that complies with medical diagnostic laboratory standards.
If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/or authorized representative and competent authority.

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. Reagents 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, instructions for use and in the safety data sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or 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. Conn, J. (1977): Biological Stains, 9th ed. Baltimore: Williams and Wilkins Co.
2. Carson, F. L., Hladik, C. (2009): Histotechnology: A Self-Instructional Text, 3rd ed., Chicago: ASCP Press
3. Lillie, R. D., Ashburn L. L., (1943): Supersaturated solutions of fat stains in dilute isopropanol for demonstration of acute fatty degeneration not shown by Herxheimer's technique, Archives of Pathology 36, 432

Warnings and precautions regarding the materials contained in the product:
Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

S3-IFU_ENV4, 13.04.2026., IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
4	Revised acc. to Regulation (EU) 2017/746 - IVDR	13.04.2026.