

SPERM-DIFF RTU KIT



IVD *In vitro* diagnostic medical device

Classification according to Regulation (EU) 2017/746 – **Class A** device

Rapid Ready-to-Use staining kit for sperm morphology analysis

Contains a fixative and red and blue components for rapid and effective staining

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30708STARVF		
EMDN code	W01030708		
REF	Catalog number	Volume	UDI-DI
SP-RTU-100		3x100 mL	03858890005875



Intended use and test principle

BioGnost's Sperm-Diff RTU kit enables rapid, simple, and high-quality staining of sperm based on the May Grünwald-Giemsa (Romanowsky) staining principle for optimal sperm morphology analysis. Sperm staining is based on a modified Romanowsky principle of differential staining using an acidic (eosin) and basic (thiazine) staining reagents following smear fixation. Sperm-Diff 1 RTU reagent is a fixative that fixes the protein and membrane structures of sperm cells, making them suitable for binding eosin and thiazine dyes. In the next step, Sperm-Diff 2 RTU reagent is applied, which contains an acidic dye, Eosin Y, that binds to basic cellular components (cytoplasmic proteins), causing the cytoplasm and certain sperm structures to take on an eosinophilic pink-red color. Sperm-Diff 3 RTU reagent contains basic dyes from the thiazine dye group (methylene blue, azures) that bind to acidic cellular regions (nucleic acids and other anionic structures), causing nuclei and some intracellular details to take on a basophilic blue or purple color. The interaction of eosin, thiazine dyes, and DNA produces characteristic Romanowsky shades of purple that allow clear differentiation of the sperm head, midpiece, and tail, as well as other cells present in the ejaculate (e.g., leukocytes, immature germ cells). The advantages of the Sperm-Diff RTU kit are: rapid staining, convenience, and simplicity due to polypropylene leak-proof containers filled with 100 mL of reagent into which slides are directly immersed, sufficient for 100-200 tests.

Product description

- **SPERM-DIFF RTU KIT** – Kit for rapid and effective staining and analysis of sperm morphology

The kit contains:	100–200 tests (SP-RTU-100)	Storage temperature
Sperm-Diff 1 RTU reagent	100 mL (SP1-RTU)	15-25°C
Sperm-Diff 2 RTU reagent	100 mL (SP2-RTU)	15-25°C
Sperm-Diff 3 RTU reagent	100 mL (SP3-RTU)	15-25°C

Additional reagents and materials that can be used in the method:

- Micropipette
- VitroGnost slides and coverslips for use in histopathology and cytology
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade

Test sample

- fresh ejaculate sample

NOTE

Close each container tightly after use to reduce the possibility of evaporation. Sperm-Diff 1 reagent is a methanol-based fixative that is highly hygroscopic and requires more frequent replenishment, especially in countries with high air humidity.

Always drain the slide on filter paper each time before immersing it in Sperm-Diff 2 or Sperm-Diff 3 RTU reagent!

Slide staining procedure

1.	Prepare a glass slide on which a thin smear of the sperm sample will be made	
2.	Prepare the smear according to the standard method used in your laboratory or follow the method below:	
	1. Transfer 20 µL of ejaculate onto a labeled glass slide using a pipette, forming a line along the center of the slide	
	2. Cover the slide with another glass slide so that the drop spreads evenly between them. Separate the slides by pulling them horizontally in opposite directions, resulting in two test slides	
	3. Air dry the smear	
3.	Immerse the slide in Sperm-Diff 1 RTU reagent	at least 1 minute
4.	Drain excess reagent from the slide onto filter paper	
5.	Immerse the slide in Sperm-Diff 2 RTU reagent	10 x 1 second
	Note: extend the incubation time if you want a stronger shade of red/purple on the slide	12 x 1 second
6.	Drain excess reagent from the slide onto filter paper	
7.	Immerse the slide in Sperm-Diff 3 RTU reagent	10 x 1 second
	Note: reduce the incubation time if you want a stronger shade of red/purple on the slide	8 x 1 second
8.	Drain excess reagent from the slide onto filter paper	
9.	Briefly rinse the slide with distilled/demineralized water	
10.	Air dry the slide	

Note: if you wish to preserve the slides, apply an appropriate type of BioMount medium (e.g. BioMount DPX) to the dried slide and cover with a coverslip.

Examine the slide under microscopic magnification of 40x or under an immersion objective (100x magnification).

Result

Sperm head; nucleus – purple
acrosome – pink
Sperm neck – dark pink
Sperm tail – light pink

Report the following as percentage:

- anomalies of sperm structural parts: head, neck, and tail
- agglutinates
- leukocytes, erythrocytes, and cells
- normal sperm forms

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this 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 staining process and diagnosis can only be carried out by authorized and professionally 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. Bertolotto, M. et Trombetta, C. (2012): *Scrotal Pathology*, Springer Heidelberg Dordrecht London New York
2. Giemsa, G. (1922): *Das Wesen der Giemsa-Färbung, Zentralb f Bakt*; 89, pp.99-106.
3. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice*, 4th ed., Bloxham, Scion Publishing Ltd.
4. May, R. et Grünwald L. (1909): *Über die Färbung von Feuchtpreparaten mit meiner Azur-Eosine methode*, Deutsche med Xschr, 35, pp.1751-1752.

Warnings and precautions regarding the materials contained in the product:

	H226	Flammable liquid and vapour.
	H301+H311+H331	Toxic if swallowed, in contact with skin, or if inhaled.
	H370	Causes damage to organs (eyes).
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P233	Keep container tightly closed.
	P280	Wear protective gloves/protective clothing/eye protection/face protection.
	P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/doctor.
	P302+P352	IF ON SKIN: wash with plenty of water.
	P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
	P308+P311	IF exposed or concerned: Call a POISON CENTER/doctor.

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 Manufacturer	 Batch code	 Consult instructions for use	 Contains sufficient for <n> tests
 Date of manufacture	 Catalogue number	 Caution	 European conformity
 Use-by date	 Temperature limit	 In vitro diagnostic medical device	 Unique device identifier

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Version	Description / reason for change	Date
2.	Revised acc. to Regulation (EU) 2017/746 - IVDR	30.06.2026.