BIOGNOST®

SPERM-DIFF RTU KIT

IVD *In vitro* diagnostic medical device CE

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

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

INSTRUCTIONS FOR USE

REF Product code: SP-RTU-100 (3 x 100 mL)

Introduction

BioGnost's Sperm-Diff RTU kit enables rapid, simple and high quality staining of sperm according to May Gruenwald-Giemsa staining method that enables quality sperm morphology analysis. Sperm-Diff RTU kit advantages include: rapid staining, practical use and simplicity due to **leakproof polypropylene** staining jars prefilled with 100 ml of reagent that enable direct immersion of samples, sufficient for **100-200** tests. Each component of Sperm-Diff kit is stabilized separately and prepared according to the highest standards.

Product description

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

The kit contains:	**100-200 tests (SP-RTU-100)**
Sperm-Diff 1 RTU reagent	100 mL (SP1-RTU)
Sperm-Diff 2 RTU reagent	100 mL (SP2-RTU)
Sperm-Diff 3 RTU reagent	100 mL (SP3-RTU)

Sperm staining procedure

NOTE: close each jar after use in order to avoid the possibility of evaporation. Sperm-Diff 1 reagent is a fixative based on methanol that is very hygroscopic and it requires more frequent filling, especially in countries situated in the region with high percentage of air humidity.

It is mandatory to decant the section on filter paper before immersing it into Sperm-Diff 2 or Sperm-Diff 3 reagent!

1.	Prepare a glass slide that will contain a thin smear of the stained sperm sample	
2.	Smear the sample on the slide	
	Note: smear the sample according to the standardized method used in the laboratory or according to the following instructions:	
	1. Transfer 20 µL of ejaculate onto the marked glass slide using the pipette and form a line on the center of the slide	
	2. Cover the slide using another glass slide so the drop spreads evenly. Separate the slides by pulling them horizontally in opposite directions, thus creating two test slides	
	3. Dry the preparation	
3.	Immerse the section into Sperm-Diff 1 RTU reagent	at least 1 minute
4.	Decant the excessive reagent from the section onto filter paper	
5.	Immerse the section into Sperm-Diff 2 RTU reagent	10 x 1 second
	Note: extend the incubation period if a stronger hue of red/purple is required	12 x 1 second
6.	Decant the excessive reagent from the section onto filter paper	
7.	Immerse the section into Sperm-Diff 3 RTU reagent	10 x 1 second
	Note: decrease the incubation period if a stronger hue of red/purple is required	8 x 1 second
8.	Decant the excessive reagent from the section onto filter paper	
9.	Rinse the section shortly using distilled (demi) water	
10.	Dry the preparation	

Note: if you wish to keep the sections, apply a suitable BioMount medium on the dried sample (such as BioMount DPX) and cover it with a glass slide

Result

Head; nucleus - purple
acrosome - pink
Midpiece - dark pink
Tail - light pink

<u>The following must be displayed as percentage:</u>

- anomalies of sperm strucural elements: head, midpiece, and tail
- agglutinates
- leukocytes, erythrocytes, and cells
- normal shapes of sperm cells

Note

Time periods of staining processes are not entirely standardized in clinical and laboratory practical experience. Time periods specified in the instruction approximately correspond to a longtime work practice with optimal results. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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 and application. 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

Keep Sperm-Diff RTU kit in a tightly closed original package at temperature between 15°C and 25°C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's 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-Farbung, *Zentralb f Bakt*; p89. 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 Farbung von Feutchpraparaten mit meiner Azur-Eosine methode,* Deutsche med Xschr, 35, str. 1751-1752.

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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	CE
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						