BIOGNOST®

EOSIN AND NIGROSIN VITAL KIT

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 - **Class A** device

Two-reagent kit for rapid detection of sperm vitality

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
ENVK-K-30	2x30 mL	03858890003666
ENVK-K-100	2x100 mL	03858890009439

Intended use and test principle

BioGnost's Eosin and Nigrosin Vital Kit is used for determining sperm vitality in ejaculate, by enabling differentiation between live and dead sperm based on the integrity of the plasma membrane. During staining of the sample, the acidic vital dye Eosin Y enters sperm with a damaged plasma membrane and stains their heads red or pink, while vital sperm with an intact membrane exclude eosin and remain unstained or only faintly pink. Nigrosin creates a dark background, providing a clear contrast between stained (non-vital) and unstained (vital) sperm when observed under a light microscope.

Product description

- **EOSIN AND NIGROSIN VITAL KIT** – Kit for determining sperm vitality

The kit contains:	**ENVK-K-30 (2 x 30 mL)**	**ENVK-K-100 (2 x 100 mL)**	**Storage temperature**
Eosin Y, isotonic solution	30 mL (EIO-OT-30)	100 mL (EIO-OT-100)	15-25°C
Nigrosin, isotonic solution	30 mL (NIO-OT-30)	100 mL (NIO-OT-100)	15-25°C

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

- 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
- BioGnost's immersion media such as Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Micropipette

Test sample

- fresh ejaculate sample

NOTE

Apply the reagent so that it completely covers the smear.

Slide staining procedure

1.	Shake the bottle of Nigrosin, isotonic solution well
2.	Mix 50 µL of ejaculate and 2 drops of Eosin Y, isotonic solution
3.	After 30 seconds, add 3 drops of Nigrosin, isotonic solution and mix well
4.	Leave for 30 seconds at room temperature
5.	Prepare a glass slide on which a thin smear of the stained sample will be made
6.	Prepare the smear according to the standard method used in your laboratory or follow the method below:
	1. Transfer 20 µL of stained 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
7.	Cover the slide with a coverslip before the smear dries and examine immediately under the microscope
	Note: if you wish to preserve the preparations, apply an appropriate type of BioMount medium (e.g. BioMount DPX) to the dried slide and cover with a coverslip
8.	Examine the slide under microscopic magnification of 40x or under an immersion objective (100x magnification)
	Note: the image at immersion magnification of 100x will very clearly show the difference between stained and unstained sperm
9.	Count 200 sperm; unstained sperm are classified as live, while red or pink sperm are classified as dead. Sperm stained only in the neck region are classified as live

Result

Live sperm – unstained and faintly pink in the neck region
Dead (damaged) sperm – red, pink

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 the 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 in 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. Björndahl, L. et al.(2004): Why the WHO Recommendations for Eosin-Nigrosin Staining Techniques for Human Sperm Vitality Assessment Must Change, Journal of Andrology, Vol.25, No.5
2. Mortimer, D. (1985): The male factor in fertility.Part 1: semen analysis.In: *Current* Problems *in Obstetrics, Gynecology and Fertility.*Vol VIII.Chicago, Ill: Year Book Medical Publishers; 75-76
3. World Health Organization (1999): WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interactions, 4th ed., Cambridge, United Kingdom: Cambridge University Press

Warnings and precautions regarding the materials contained in the product:	
	H317 May cause an allergic skin reaction. P280 Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352 IF ON SKIN: wash with plenty of water/… P308 + P313 IF exposed or concerned: Get medical advice/attention.

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

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