BIOGNOST®

EOSIN-NIGROSIN VITAL

IVD *In vitro* diagnostic medical device CE

Fast detection of sperm vitality with one reagent
INSTRUCTIONS FOR USE

REF Product code: ENV- 30 (30 mL) ENV-100 (100 mL)

Introduction

BioGnost's Eosin and nigrosin vital is used for detection of sperm vitality. Using the reagent is very fast (one step) and simple. During sample staining eosin Y dye enters dead sperm cells (cells with damaged plasma membrane) and stains them red. Nigrosin dye provides dark contrast for better visualization of living, non-stained sperm cells.

Product description

- **EOSIN-NIGROSIN VITAL** – Reagent for detection of sperm vitality

Testing sample

- Fresh ejaculate sample

Other necessary preparations:

- Micropipette
- Eppendorf test tube
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of VitroGnost glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, and BioMount C
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and B

Staining procedure

1.	Shake Eosin-nigrosin vital reagent bottle well
2.	Mix equal volumes of Eosin-nigrosin vital reagent and ejaculate sample (for example, mix 50 μL of Eosin and nigrosine reagent with 50 μL of ejaculate in Eppendorf test tube)
3.	Mix well
4.	Let it set for 30 seconds at room temperature
5.	Prepare a glass slide that will contain a thin smear of the stained sample
6.	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 the stained ejaculate sample 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
7.	Dry the sections
	Note: if you wish to keep the sections, apply a suitable BioMount medium on the sample (such as BioMount DPX) and cover it with a glass slide
8.	Study the sample under microscopical magnification factor of 40x or under immersion lens (100x magnification factor)
	Note: image under immersion lens 100x magnification will very clearly show the difference between stained and non-stained sperm cells
9.	Count 200 sperm cells; non-stained are classified as living sperm cells, while those stained red or pink are classified as dead sperm cells. Sperm cell that only has its neck stained is classified as living.

Result

Living sperm - non-stained and stained in the area of neck

Dead (damaged) sperm - red, pink

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. Reagents 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 Eosin-nigrosine vital 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. 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 Interacions, 4th ed., Cambridge, United Kingdom,: Cambridge University Press

ENV-X, V2-EN2, 3 August 2017, AK/VR

Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	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					