

EOSIN AND NIGROSINE VITAL KIT

IVD *In vitro* diagnostic medical device



Kit for rapid detection of sperm vitality

INSTRUCTIONS FOR USE

REF Product code: ENVK-K-30 (2 x 30 mL) ENVK-K-100 (2 x 100 mL)

Introduction

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

Product description

- **EOSIN AND NIGROSINE VITAL KIT** – Kit for detection of sperm vitality

| The kit contains: | ENVK-K-30 (2 x 30 mL) | ENVK-K-100 (2 x 100 mL) |
|------------------------------|-----------------------|-------------------------|
| Eosin Y, isotonic solution | 30 mL (EIO-OT-30) | 100 mL (EIO-OT-100) |
| Nigrosine, isotonic solution | 30 mL (NIO-OT-30) | 100 mL (NIO-OT-100) |

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 the Nigrosin, isotonic solution bottle well |
| 2. | Mix 50 μ L of ejaculate and 2 drops of Eosin Y, isotonic solution |
| 3. | After 30 seconds, add 3 drops of Nigrosine, isotonic solution and 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 μ L 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. | Cover the sample with a glass slide before smear gets dried and immediately study it under the microscope |
| | 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 |
| 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 and nigrosine vital 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. 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

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|  | Refer to the supplied documentation |  | Storage temperature range |  | Number of tests in package |  | Product code |  | European Conformity |
|  | Refer to supplied instructions |  | Keep away from heat and sunlight |  | Valid until |  | Lot number |  | Manufacturer |
|  | For <i>in vitro</i> diagnostic use only |  | Keep in dry place |  | Caution - fragile | | | | |

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