



vitalTest[®]
Kit **REF** HT - VT100
for 100 determinations



Assay for sperm quality assessment

Principle of the method

Sperm vitality is a routine assay for sperm quality assessment. Vital stains differentiate live from dead sperm, based on the integrity of the plasma membrane. Intact plasma membrane is able to exclude certain stains either for bright-field microscopy or fluorescence microscopy. Sperm vitality evaluated with the combined use of fluorescent dyes correlates with that determined by eosin-nigrosin stains (bright-field microscopy) but opens a new range of possibilities for automatic counting using flow cytometry or fluorescence microscopy and image analysis data collection.

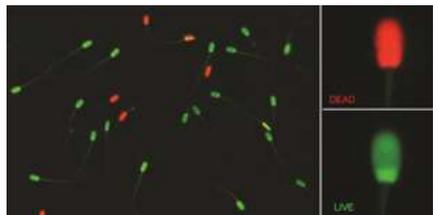
The vitalTest[®] kit can be used in fresh or unfrozen semen samples, for standard semen analysis in routine assessment of male fertility.

The kit provides a useful tool for monitoring sperm survival under varying experimental conditions of sperm samples under stress. It has been designed for use under fluorescence microscopy but the results might also be analysed using flow cytometry.

Main characteristics

vitalTest[®] provides a High Effective Fluorescent Signal for confident visual analysis.

- **High sensitivity:** Detects low levels of nucleic acid in sperm.
- **Less time consuming:** Simple protocol; single incubation; simultaneous visualization of two fluorochrome emissions
- **High resolution:** Clearer images with higher contrast.



Each kit contains

The kit provides two reagents to evaluate sperm vitality by a dual emission fluorescent signal (Green: Live; Red: dead).

PI, red solution 120 µl
AO, green solution 120 µl

Protocol

Step 1: Dilute the sperm sample to a final concentration of 10-15 million per millilitre in appropriate diluents (variable among species).

Step 2: Add 10 µl of the diluted sperm sample onto a clean slide. Add 1 µl of AO, and 1 µl of PI to the diluted sperm sample and mix.

Step 3: Cover with a clean coverslip and observe under the fluorescence microscope.

Fluorescence spectral characteristics

Both single-band or dual (green/red) fluorescence bandpass filters could be used. The vitalTest kit includes Propidium Iodide (ex503-530/em640) and Acridine Orange (ex536/em617) solutions stabilized for long lasting. Single-long pass band (ex500/em600-640) is also recommended for simultaneous visualization.

Score a minimum of 300 sperm per sample following the criteria: "green" as live cells and "red" as dead cells. In some cases, "green-red" fluorescent sperm nuclei are observed. This is mainly due to an incipient loss of membrane integrity. Score this sperm nuclei as "dead".

Precautions

The vitalTest® kit contains fluorochromes which bind directly to DNA. Acridine derivatives are potentially teratogenic. Use gloves for handling and dispense residues to special container.

Storage conditions

Store at 4°C.

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