



DUO-VITAL
KIT FOR SPERM VITALITY ASSESSMENT
The easy way to analyze live/dead sperm cells
For research purpose only
Do not use for diagnosis purpose

Application

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

Principle of the method:

Sperm vitality is a routinary 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 *DUO-VITAL kit* can be use in fresh or unthaw semen samples.

The *DUO -VITAL kit* is intended for standard semen analysis in routine assessment of male fertility.

The *DUO -VITAL kit provides* a useful tool for monitoring sperm survival under varying experimental conditions of sperm samples under stress.

The *DUO-VITAL kit* has been designed for use under fluorescence microscopy but the results might also be analyzed using flow cytometry.

Main characteristics:

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.

Kit content:

- Reagent A, red solution (acridine orange) 100 µl
- Reagent B, green solution (propidium iodide) 100 µl

Instruction for use

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 reagent A, and 1 µl of reagent B to the diluted sperm sample and mix.

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

Microscopic visualization

DUO-VITAL provides a High Effective Fluorescent Signal for confident visual analysis

Fluorescence spectral characteristics

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

Score "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".

Caution: The *DUO -VITAL kit* contains fluorochromes which bind directly to DNA. Acridine derivates are potentially theratogenic. Use gloves for handling and dispense residues to special cont

