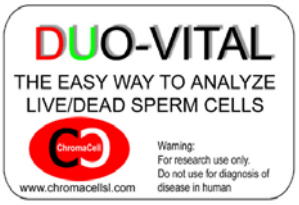


DUO-VITAL

KIT FOR SPERM VITALITY ASSESMENT IN ANIMAL SPECIES

The assessment of sperm vitality is one of the basic elements of semen analysis. Vital stains can help to differentiate live from dead sperm. Results of vitality test are linked to the integrity of the plasma membrane of viable sperm; an 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 citometry or fluorescence microscopy and image analysis data collection.

The *DUO-VITAL kit* is for the evaluation of sperm vitality from fresh or unthaws semen samples. The kit provides the user with specific adjusted solutions of two reagents to evaluate sperm vitality by means of a dual emission fluorescent signal (Green: Live; Red: dead).
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 citometry.

Main characteristics of DUO-VITAL

High sensitivity: Detects low levels of nucleic acid in sperm.

Less time consuming: Simple protocol; a single instant incubation for a simultaneous visualization of two fluorochrome emissions. No separate incubations are necessary.
High resolution: Clearer images with higher contrast.

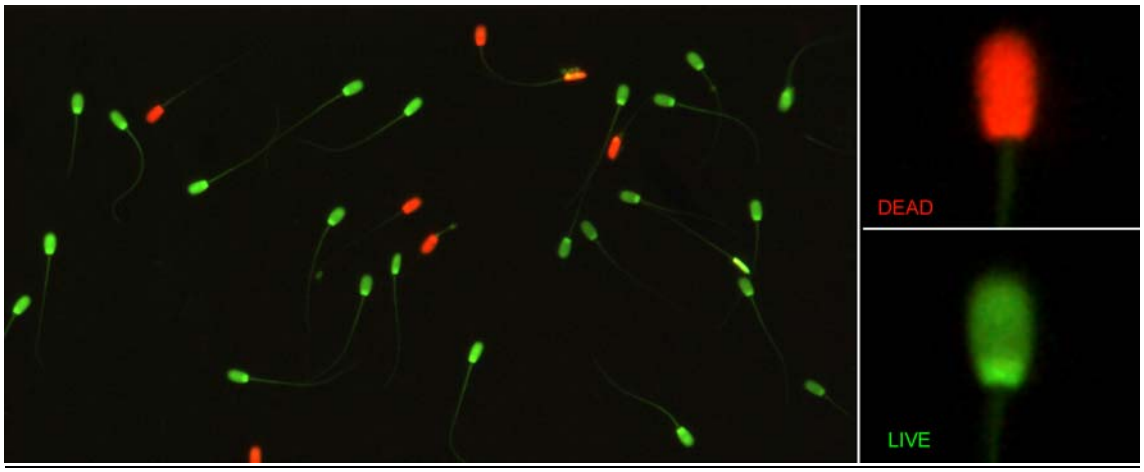
Mode of use

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

Step 2: Add 15 microliters of the diluted sperm sample onto a clean slide. Add 1 microliter of the fluorochrome solution red, and 1 microliter of the fluorochrome solution green to the diluted sperm sample and mix. Cover with a clean coverslip and observe under the fluorescence microscope.

Score “green” as live cells and “red” as dead cells. In some case, “gree-red” fluorescent sperm nuclei are observed. This is mainly due to an incipient loss of membrane integrity. Score this sperm nuclei as “dead”.

Used under these conditions, *DUO-VITAL* provides a High Effective Fluorescent Signal for confident visual analysis of sperm vitality. The combination of DUO-VITAL with Computer Assisted Sperm Analysis - CASA/Leja (or alternatives) multiple chamber slides allows the simultaneous assessment of sperm concentration, vitality and motility. A CASA system for such purposes is available (SCA-Vital. Microptic SL, Barcelona, Spain).



For fluorescence analysis, both single-band or dual (green/red) fluorescence bandpass filters could be used. The *DUO -VITAL* kit includes Acridine Orange (503/530/640) and Propidium Iodide (536/617) based solutions stabilized for long lasting. Single-long pass band (excitation 500; emission 600/640) is also recommended for simultaneous visualization.

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 containers.

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