FluoCount Protocol

KIT FOR SPERM MOTILITY AND CONCENTRATION ASSESSMENT

UNDER FLUORESCENCE IN HUMAN AND ANIMAL SPECIES

The assessment of sperm motility and concentration is one of the basic elements in semen analysis.

FluoCount kit enables the evaluation of motility and concentration in fresh or thawed semen samples containing high percentage of debris, mainly due to the use of egg yolk or milk based extenders. The kit enables to differentiate spermatozoa from the extender debris and is intended for standard semen analysis in routine assessment or research studies of male or animal fertility.

FluoCount is composed by a fluorescent dye and may be used in fluorescence microscopy.

200 µl/kit = 200 analyses

Storing conditions: Solutions should be stored at 2-4°C degrees and protected from light.

Caution: the stain solution is mutagen and must be handled with care. The dye must be disposed of safely and in accordance with applicable regulation.

High sensitivity: The dye detects low levels of nucleic acid in sperm.

Reagents for in vitro diagnostic use only: The **FluoCount** includes Hoechst 33342, Trihydrochloride Trihydrate (330/380) based solution stabilized for long lasting.

Microscope filter: **DAPI filter** (EX 330-380, DM 400, BA 420, standard filter for UV).

Procedure

Step 1: use two empty eppendorf tubes to prepare the following working solutions for the assessment: For **A eppendorf** take 250 µl of PBS and add 1 µl of **FluoCount**.

For **B eppendorf** take 50 µl of PBS.

Incubate these two working solutions at 37°C for 5 minutes.

Step 2: place100 µl of semen sample (fresh or thawed), stored at 37°C, into the B eppendorf.

Ensure that the semen **sample has a maximum concentration** of 50M/ml and make the appropriate dilutions with PBS. Otherwise, the concentration is too high to distinguish individual cells.

Step 3: add 50 µl of the A working solution into the vial B.

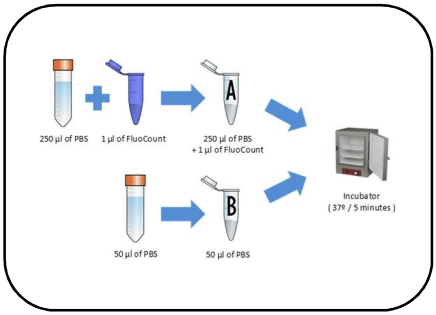
Step 4: leave the **final B eppendorf** tube solution (containing 50 µl of PBS + 100 µl of semen + 50 µl of A eppendorf working solution) in the incubator at 37°C for a minimum of 5 minutes, or until **BLUE** fluorescent dye could be correctly detected.

Step 5: fill a 20 microns counting chamber with a small aliquot of the final vial B solution and analyze under fluorescence microscope with DAPI filter.

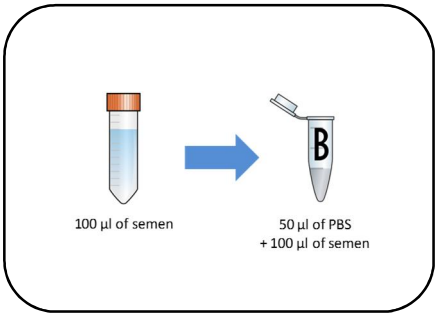
Ready for the automatic assessment with the CASA system Sperm Class Analyzer - SCA. (www.micropticsl.com).

fig 1: automatic assessment with the SCA

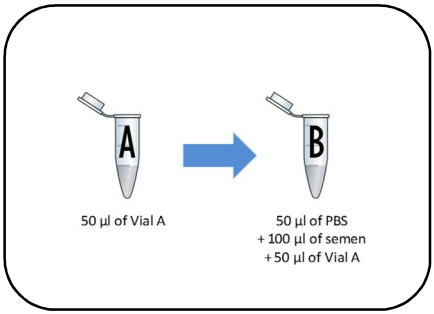
Step 1:



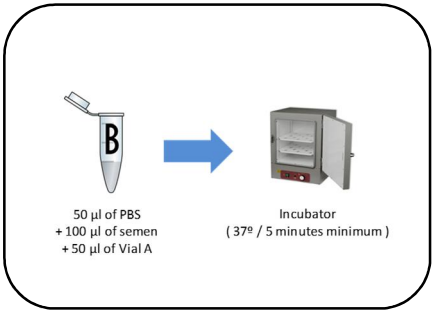
Step 2:



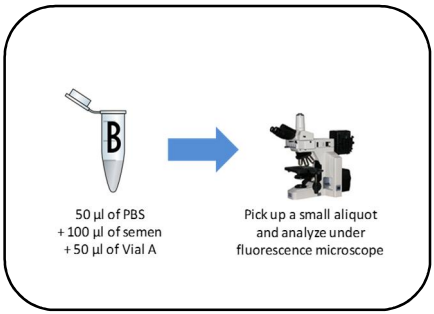
Step 3:



Step 4:



Step 5:



Distributed by:

Microptic S.L.
Av. Josep Tarradellas, 8, 1º 6ª
08029 Barcelona (Spain)
Tel. +34 93 419 29 10
Fax +34 93 419 94 26
www.micropticsl.com