FluoCount Protocol for bull

KIT FOR BULL SPERM MOTILITY AND CONCENTRATION ASSESSMENT

UNDER FLUORESCENCE

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 bull fertility.

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

400 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 one empty eppendorf tube to prepare the following working solution for the assessment:

For **A eppendorf** take 100 µl of PBS and add 2 µl of **FluoCount**.

Incubate this solution at 37°C for 5 minutes.

Step 2: then place100 µl of semen sample (fresh or thawed), stored at 37°C, into the same A eppendorf tube and mix it carefully or vortex during 5 seconds.

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: leave the **final A eppendorf** tube solution (containing 100 µl of PBS + 100 µl of semen + 2 µl of **FluoCount**) in the incubator at 37°C. Incubation times are different for fresh and thawed samples:

For fresh samples a minimum of 10 minutes.

For thawed samples a minimum of 20 minutes.

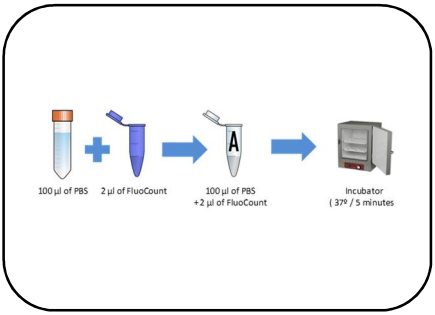
Or wait until **BLUE** fluorescent dye could be correctly distinguished.

Step 4: fill a 20 microns counting chamber with a small aliquot of the final vial A solution and analyze under fluorescence microscope with DAPI filter.

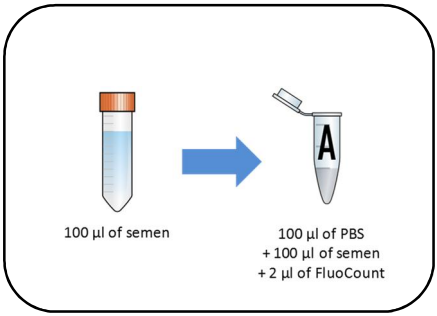
Ready for the automatic assessment with the CASA system Sperm Class Analyzer - SCA. (www.micropticsl.com).

fig 1: automatic assessment with the SCA

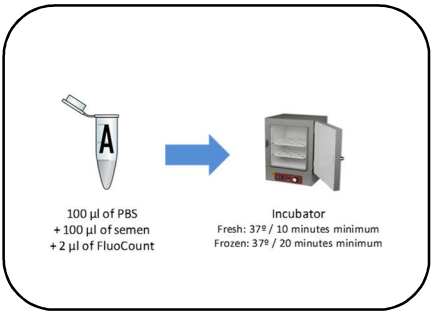
Step 1:



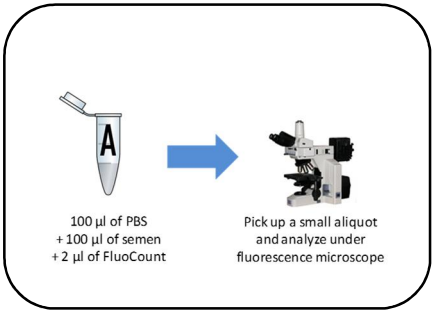
Step 2:



Step 3:



Step 4:



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