VIADENT STAIN INSTRUCTIONS –Version 12
ANIMAL VIABILITY ANALYSIS

Equipment Required

Two pipettes, one 100 - 1000 µl, second 50-200 µl
Incubator @ 37°C
Clean microcentrifuge tube
5 ml test tube
Clear, low lipid content, culture medium (EXCEL, PBS, etc.)
VIADENT Stain Tube
Adjustable Speed Vortex
Timer

IVOS Setup

1) On INFO screen, set SAMPLE:DILUENT to 1:1 and enter the total VOLUME of semen sample.
2) Select the ANALYSIS SETUP appropriate for VIADENT analysis.
3) On the STAGE SETUP screen, select VIADENT AUTO.

Staining Protocol

1) *Stain Solution:* Pipette 1000µl of culture medium into the VIADENT STAIN tube (This gives a stain concentration of 40 µg/ml). Vortex slowly for 5 seconds. Add 1 ml of stain solution to 3ml of culture medium (This gives a stain concentration of 10 µg/ml). Vortex slowly for 5 seconds. Maintain at 37°C.

2) *Sample:* Prepare a properly diluted semen sample at a concentration of 20-60 M/ml.

3) *Staining:* Add 500 µl of diluted semen sample to 500 µl of stain solution to create the final stain concentration of 5µg/ml.
4) *Incubation:* Incubate the stained sample at 37°C for 2 minutes.
5) *Analysis:* The sample is now ready for analysis with blue light and VIADENT filter block.

Notes

If sperm are not detected on the PLAYBACK screen (sperm image is focused but too faint) the staining level may be too low. To improve the staining level, increase the VIADENT stain concentration.

If the concentrations of sperm are too high and dilutions are required, it is *essential* to discard the stained sample, prepare a new sample, and stain that.

Adjustments in staining concentration and/or staining time may be necessary due to differences in individual samples or types of extender.

STORAGE

Store VIADENT Stain tubes in the refrigerator.
Avoid excessive exposure of VIADENT Stain tubes to light.

REAGENTS

40 µg of stain in each tube. Contains bis Benzimide Trihydrochloride. The reagent included in the reaction vials is for laboratory use only and not for household or other uses.
The toxicological properties of bis Benzimide Trihydrochloride have not been thoroughly investigated. Exercise due care.

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