

VIADENT STAIN INSTRUCTIONS

CASA II and Legacy CASA V12 & V14

Equipment Required

- Two pipettes, one 100 - 1000 µl, one 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 II / IVOS Setup

1. Select a setup configured for VIADENT analysis.
2. Enter the appropriate dilution (1:1 for neat samples) and volume of semen sample.
3. **For Version 12 & 14:** On the STAGE SETUP screen, select VIADENT AUTO.

Staining Protocol

1. *Prepare Stain Stock Solution:*
 - a. **Option 1:** This gives a stock stain concentration of 10 µg/ml. Pipette 1000µl of culture medium into the VIADENT STAIN tube (This gives an initial stain concentration of 40 µg/ml). Vortex slowly for 5 seconds. Add 1 ml of stain solution to 3ml of culture medium. Vortex slowly for 5 seconds. Maintain at 37°C.
 - b. **Option 2:** This gives a stock stain concentration of 20 µg/ml. Pipette 1000µl of culture medium into the VIADENT STAIN tube (This gives an initial stain concentration of 40 µg/ml). Vortex slowly for 5 seconds. Add 1 ml of stain solution to 1ml of culture medium. 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:*
 - a. **Option 1:** Add 500 µl of diluted semen sample to 500 µl of stain solution to create the final stain concentration of 5µg/ml.
 - b. **Option 2:** Add 500 µl of diluted semen sample to 500 µl of stain solution to create the final stain concentration of 10 µg/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

- *Stain Stock Solution Option 1 is recommended for most fresh or cooled samples.*
- *Stain Stock Solution Option 2 is recommended for samples in cryoprotectant media and bovine sperm samples.*
- *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 that the stained sample be prepared from an aliquot of the diluted sample. This dilution must be allowed for by entering in the Dilution ratio. See the section on Sequential Dilutions in the Software Manual.*
- *Adjustments in staining concentration and/or staining time may be necessary due to differences in individual samples or types of extender.*

STORAGE

VIADENT Stain tubes should be stored at 5-10°C.
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.
Refer to the Safety Data Sheet (SDS) for additional information.

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