IDENT STAIN INSTRUCTIONS – Versions 10, 12 and 14

(see reverse side for additional protocols)

HUMAN
MOTILE SPERM ANALYSIS

Equipment Required

One 50 - 1000 µl adjustable pipet
Incubator @ 37°C
Clean microcentrifuge tube
Culture medium (Ham's F-10, B2, M-199, etc.)
IDENT Stain Tube
Adjustable Speed Vortex

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 IDENT analysis of motile sperm.
3) **For Version 10:** On the CONFIGURE STAGE screen, select IDENT Option Set B. **For Version 12 & 14:** On the STAGE SETUP screen, select IDENT FULL ANALYSIS.

Staining Protocol

1) *Stain Solution:* Pipette 1 ml of culture medium into the IDENT stain tube. Vortex briefly to mix and maintain at 37°C. This gives a stain concentration of 40 µg/ml.
2) *Sample:* Pipette 100 µl of semen sample into a clean microcentrifuge tube and maintain at 37°C. Add an additional 50 µl of culture medium to the semen sample.
3) *Staining:* Add 50 µl of stain solution to the 150 µl of diluted semen sample. This gives a final stain concentration of 10 µg/ml.
4) *Incubation:* Incubate the stained sample at 37°C for 10 minutes.
5) *Analysis:* The sample is now ready to be analyzed.

Notes

If sperm are not detected on the PLAYBACK screen (sperm are too faint) the staining level may be too low. To improve the staining level:
a) increase the incubation time to 20 minutes; or
b) use 100µl of sample, add 25 µl of medium and 75 µl of stain solution. This gives a final stain concentration of 15 µg/ml. SAMPLE:DILUENT ratio remains at 1:1 on the INFO screen.
See also Note under Bull regarding sperm concentration.

BULL
MOTILE SPERM ANALYSIS

Equipment Required

One 100 - 500 µl adjustable pipet
Incubator @ 37°C
Clean microcentrifuge tube
Clear, low lipid content, culture medium (TALP, PBS, etc.)
IDENT Stain Tube
Adjustable Speed Vortex

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 IDENT analysis of motile sperm.
3) **For Version 10:** On the CONFIGURE STAGE screen, select IDENT Option Set B. **For Version 12 & 14:** On the STAGE SETUP screen, select IDENT FULL ANALYSIS.

Staining Protocol

1) *Stain Solution:* Pipette 500 µl of culture medium into the IDENT Stain tube. Vortex slowly for 5 seconds. This gives a final stain concentration of 80 µg/ml.
2) *Sample:* Pipette 100µl of sample into a clean microcentrifuge tube and maintain at 37°C.
3) *Staining:* Add 100 µl of stain solution to the 100 µl of semen sample. This gives a final stain concentration of 40 µg/ml.
4) *Incubation:* Incubate the stained sample 37°C for 10 minutes or longer.
5) *Analysis:* The sample is now ready for analysis.

Notes

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 it on the INFO Screen during your setup prior to analysis.
For Tris extender, or high lipid based extenders like egg yolk, hydrate the IDENT pellent with 4 µl distilled water before beginning the staining protocol.
It is recommended that all extenders be treated for use with IDENT before beginning.
Adjustments in staining concentration and/or staining time may be necessary due to differences in individual samples or types of extender.

STORAGE

Store IDENT Stain tubes in the refrigerator. Avoid excessive exposure of IDENT 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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IDENT STAIN INSTRUCTIONS
(continued)

VERSION 10 TOX-IVOS
TOXICOLOGY (Cauda Homogenate)

Equipment Required

One 100 µl pipet
Distilled Water
IDENT Stain Tube
Adjustable Speed Vortex

IVOS Setup

1) On the INFO screen, set **SAMPLE:DILUENT to 1:0.0**, enter the total VOLUME of the homogenate and enter the TISSUE WEIGHT, in grams, of the cauda.
2) Select the ANALYSIS SETUP appropriate for IDENT analysis of static sperm.
3) On the CONFIGURE STAGE screen, select IDENT Option Set D (Set D implicitly includes the dilution ratio involved in the staining protocol, so the dilution ratio does not need to be entered.)

Staining Protocol

1) *Stain Solution:* Pipette 100 µl of distilled water directly into the IDENT Stain tube. Vortex to mix thoroughly. This gives a stain concentration of 400 µg/ml.
2) *Sample:* Add 100 µl of well vortexed homogenate to the distilled water in the IDENT Stain tube. Vortex slowly for 5 seconds. This gives a final *stained sample* concentration of 200 µg/ml.
3) *Incubation:* Allow the tube to stand for 2 minutes.
4) *Analysis:* The sample is now ready for analysis.

Notes

If the concentration of sperm is too high for analysis, the *stained sample* must be diluted. Dilute the stained sample with distilled water. Enter the dilution value on the INFO screen. (e.g.: If 200 µl of stained sample is diluted with 400 µl of distilled water, enter the SAMPLE:DILUENT ratio of 1:2).

VERSIONS 12 & 14 TOX-IVOS
TOXICOLOGY (Cauda Homogenate)

Equipment Required

One 100 µl pipet
Distilled Water
IDENT Stain Tube
Adjustable Speed Vortex

IVOS Setup

1) On the INFO screen, set **SAMPLE:DILUENT to 1:1**, enter the total VOLUME of the homogenate and enter the TISSUE WEIGHT, in grams, of the cauda.
2) Select the ANALYSIS SETUP appropriate for IDENT analysis of static sperm.
3) On the STAGE SETUP screen, select IDENT STATIC ANALYSIS (the dilution ratio *must* be entered on the INFO screen).

Staining Protocol

1) *Stain Solution:* Pipette 100 µl of distilled water directly into the IDENT Stain tube. Vortex to mix thoroughly. This gives a stain concentration of 400 µg/ml.
2) *Sample:* Add 100 µl of well vortexed homogenate to the distilled water in the IDENT Stain tube. Vortex slowly for 5 seconds. This gives a final *stained sample* concentration of 200 µg/ml.
3) *Incubation:* Allow the tube to stand for 2 minutes.
4) *Analysis:* The sample is now ready for analysis.

Notes

If the concentration of sperm is too high for analysis, the *stained sample* must be diluted. Dilute the stained sample with distilled water. Enter the adjusted dilution value on the INFO screen (remember to take into consideration the dilution of 1:1 that occurs in the IDENT stain tube).

Please see reverse side for storage instructions.

Hamilton Thorne, Inc.
100 Cummings Center, Suite 465E
Beverly, MA 01915
(978) 921-2050, (800) 323-0503
Fax: (978) 921-0250
sales@hamiltonthorne.com, www.hamiltonthorne.com

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