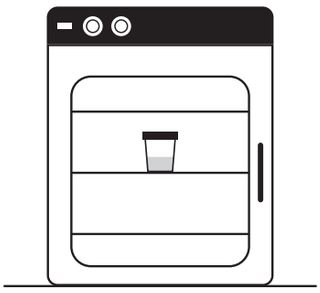
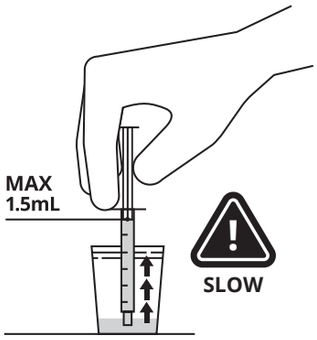
VetMotl®, Inc.

MULTI (3mL)

Bovine Sperm Separation Device | VMB3000

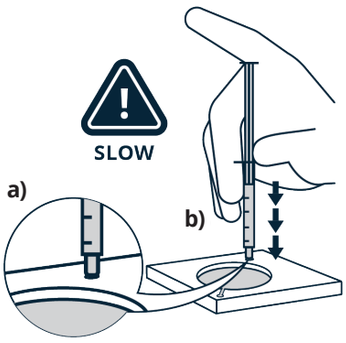
INSTRUCTIONS FOR USE
BOVINE IVF

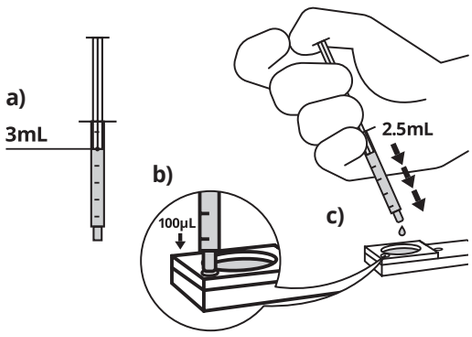




1 Fresh sample: Allow sample to liquify.
Frozen sample: Thaw in warm water bath at 35-38˚C for 1 minute.

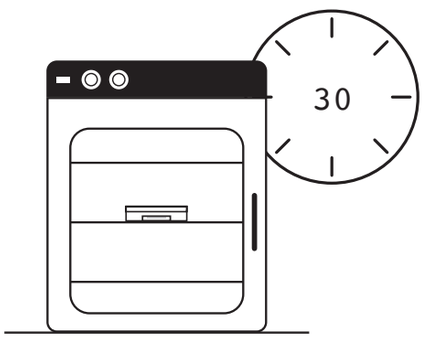
2 Fresh sample: Use 1mL syringe to draw 3mL aliquot. Thawed sample — 1.5mL volume: add 1.5mL warm media and mix gently.

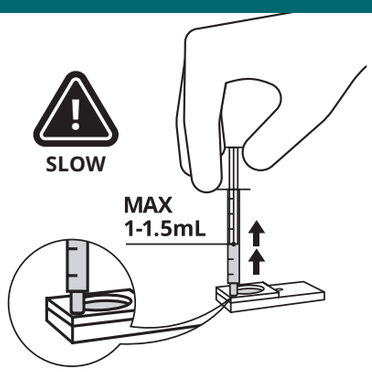




3 **a)** Achieve seal. **b)** Slowly inject sample.

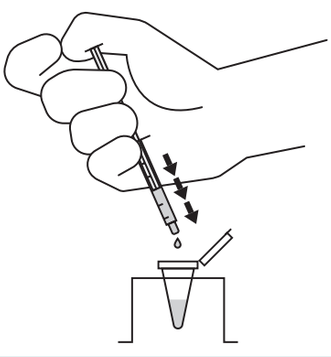
4 **a)** Draw 2.5mL of media. **b)** Prime outlet channel. **c)** Cover membrane surface.





5 Incubate at 38°C for 30 minutes.

6 Slowly aspirate 1-1.5mL.



IVF

Transfer the collected sample into a 5mL conical tube.

Centrifuge the conical tube for 5-10min at 300 x g.

Remove the supernatant, being careful to not disturb the lower pellet.

Perform count and motility as usual and dilute if needed to achieve appropriate final insemination concentration.

Store tube in a CO_2 incubator until insemination.

7 Sample Handling After Collection – **BOVINE IVF**

VetMotl Multi (3mL) Bovine Sperm Separation Device (VMB3000)

— Instructions for Use —

Important Information

- Carefully adhere to the recommended volumes for each step. Avoid over- or under-filling the device.
- Do not exceed the 30-minute incubation time.
- Keep the device level during use – do not tip or rock.
- Device is single-use only. It may not be reused.

Note on Incubation

Good tissue practices necessitate matching media to incubation conditions. If using a bicarbonate-buffered media, incubate in a humidified, 38°C, gassed incubator. If using a HEPES-buffered media, incubate in a humidified, non-gassed incubator. If no incubator with humidity is available, add a 35mm dish of deionized or distilled water, uncovered, to the Petri dish containing the device before placing the covered dish with the device and the 35mm dish into the 38°C incubator.

Preparation

1. Gather your supplies and work on a clean surface.
2. Thaw frozen semen sample at 35-38°C for 1 minute.
3. Incubate fresh semen sample at 38°C for 20-30 minutes to allow for liquefaction.
4. Carefully open the device package without touching the device membrane.

When Drawing from a Fresh Specimen

5. Use a 5ml syringe to slowly draw a 3ml aliquot of the liquefied semen specimen. If there is insufficient sample volume, add media to bring volume to 3ml.

When Drawing from a Frozen Specimen

5a. Thaw the semen straw in water at 38°C for 1 minute. Wipe the straw to dry off the water. Cut the sealed end and then cut underneath the cotton plug to allow the contents to run into a 3ml Eppendorf Tube, pre-warmed at 38°C. Tap the straw to empty it completely or use a pipette to blow air through the straw.

5b. Dilute the thawed semen 1:1 with warm media. Make sure that you have 3ml in total in the tube. If you have less, add enough media to bring the total volume to 3ml and gently mix.

5c. Use a 5ml syringe to slowly draw a 3ml aliquot of the diluted semen specimen.

Inject Sample

6. Holding the device securely, carefully insert syringe into the device Inlet Port, applying gentle pressure to achieve a firm onnection between syringe and device.
7. Apply slow and steady pressure to inject the 3ml sample. Be careful to avoid the formation of bubbles under the membrane.

Add Media

8. Prepare a fresh 5ml syringe with 2.5ml of media.
   a) Prime the Outlet Port/Concentration Chamber by injecting a small volume of media (approximately 100µl), until the media travels through the channel to the membrane surface.
   b) Disconnect the syringe from the Outlet Port and apply the remaining media in the syringe to the surface of the upper membrane by dropping from approximately 2cm above the membrane. Completely cover the upper membrane with media, making sure media touches all the edges of the upper chamber and connects with the droplet of media that was used to prime the Outlet Port/Concentration Chamber. Do not tilt the device to spread the media.

Incubate Sample

9. Place the device into a Petri dish and cover. Keep the device horizontal and covered at all times during the incubation. Incubate at 38°C for 30 minutes.

Collect Separated Sample

10. Insert a fresh 5ml syringe into the Outlet Port, achieving a firm connection. Slowly aspirate 1-1.5ml of the sperm-containing fluid.

Sample Handling after Collection

11. Centrifuge at 300 x g for 5-10 minutes and remove as much of the supernatant as possible.
12. Resuspend the pellet and calculate the concentration of spermatozoa by your preferred method.