VetMotl®, Inc.

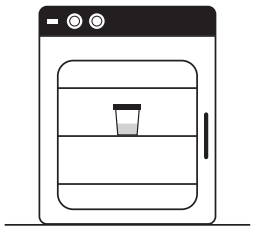
MULTI (850µL)

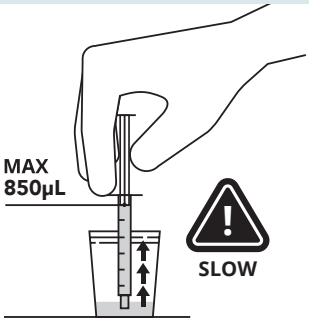
Bovine Sperm Separation Device | VMB0850

INSTRUCTIONS FOR USE

BOVINE IVF

(Refer to page 2 for further instructional details.)





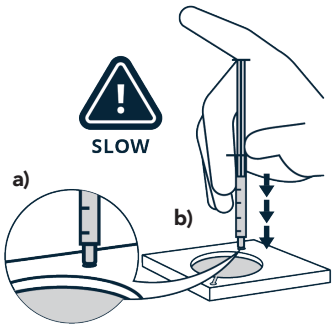
1 Equilibrate IVF medium before preparing fresh semen or thawing frozen semen. Dilute semen to practical working volume of up to 900µL.

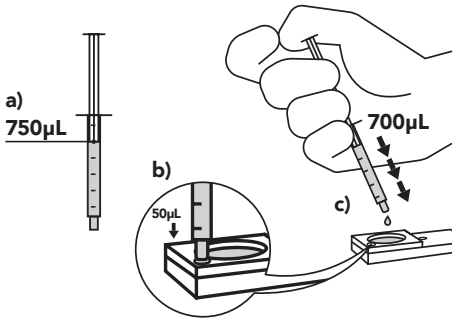
- For sex-sorted semen, use an IVF medium without PHE and heparin.
- For conventional semen, use an IVF medium with PHE and heparin to promote motility.

Gently homogenize the sample with a pipette.

2 IMPORTANT: Keep sample and media at 37°C throughout procedure.

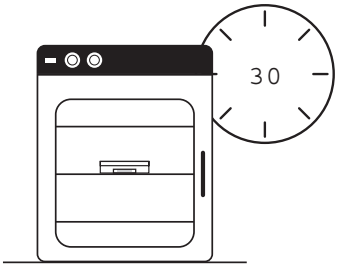
Use 1mL syringe to draw 850µL aliquot of extended semen sample from Step 1.

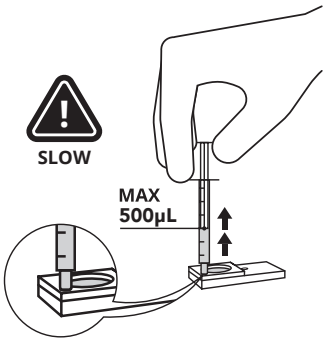


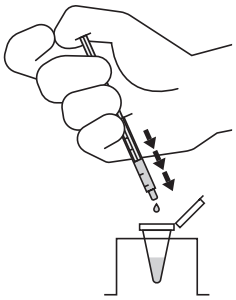


3
a) Establish secure seal in the inlet port on device.
b) Apply slow and steady pressure, delivering the 850µL sample into lower chamber through inlet channel.

4
a) Use fresh 1mL semen-safe syringe to draw 750µL of IVF medium.
b) Prime outlet channel with 50-100µL of medium.
c) Dispense balance of medium onto the surface of the upper membrane. Use caution to avoid contacting the membrane.







5 Place device in Petri dish and cover. Incubate for 30 minutes. Maintain stable working temperature and avoid significant temperature fluctuations.

6 Establish secure seal with a fresh 1mL syringe in outlet port of the device. Slowly aspirate 500µL of sperm sample from the upper chamber and place into a capped tube. Add 500µL of fresh IVF medium.

7 Centrifuge at 300 x g for 3 minutes and remove supernatant.
Calculate the concentration of sperm and re-suspend as required.
Store processed sperm until insemination.

VetMotl® Multi (850µL) Bovine Sperm Separation Device

VMB0850

– Instructions for Use –

1. Equilibrate IVF medium before preparing fresh semen or thawing frozen semen.
2. Thaw semen at 35-37°C for 45 seconds.
3. For the lower device chamber:

   Note: The volume in sex-sorted and conventional straws can vary from the labelled volume. In addition, sex-sorted and conventional bovine semen straws may use different extender formulations, which can influence sperm capacitation timing.

   a) For sex-sorted semen, add 750µL of an IVF medium without PHE and heparin.

   b) For conventional semen, add 450µL of an IVF medium with PHE and heparin to promote motility.

   Ensure that the semen in the IVF medium is diluted to a practical working volume of up to 900µL. Gently homogenize the sample with a pipette.
4. Use a 1mL semen-safe syringe to draw an 850µL aliquot of the extended semen.
5. Establish a secure seal in the inlet port, then apply slow, steady pressure to inject the 850µL sample into the lower chamber through the inlet channel.
6. For the upper device chamber: Use IVF medium for the sex-sorted or conventional semen sample as described in Step 2.

   a) Use a fresh 1mL semen-safe syringe to draw 750µL of medium.

   b) Prime the outlet port with 50–100µL of the medium.

   c) Dispense the balance of the medium onto the surface of the upper membrane by dropping from approximately 2cm above the membrane. Completely cover the upper membrane with medium.

   d) Do not tilt the device to spread the medium. Gently tease out any bubbles in the medium with the syringe, being careful not to contact the membrane.
7. Place the device in a Petri dish and cover. Incubate for 30 minutes, maintaining the sample at a stable working temperature and avoiding significant temperature fluctuations. Use your normal IVF incubator temperature (*e.g.*, 38.5–38.7°C in 5% CO_2 with bicarbonate medium).
8. Insert a fresh 1mL syringe into the outlet port, establishing a firm connection. Slowly aspirate 500µL of the sperm-containing fluid from the upper chamber and place it into a capped tube. *Note:* Removing more than 500µL risks aspirating suboptimal sperm and debris from beneath the membrane, compromising the integrity of the aspirated sample.
9. Add 500µL of fresh IVF medium to the aspirated sample in the capped tube.
10. Centrifuge at 300 x g for 3 minutes and then gently remove the supernatant.
11. Leave a 30µL pellet. Calculate the sperm concentration and resuspend the pellet in fresh medium.
12. Store processed sperm at appropriate pH and temperature until insemination.
13. Add sperm to the IVF system according to your protocol; for example, to a final concentration of $1x10^6$ sperm/mL or 2,000–25,000 sperm/oocyte.