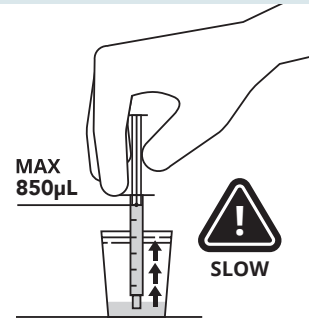
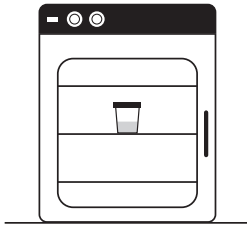


## INSTRUCTIONS FOR USE BOVINE IVF

(Refer to page 2 for further instructional details.)



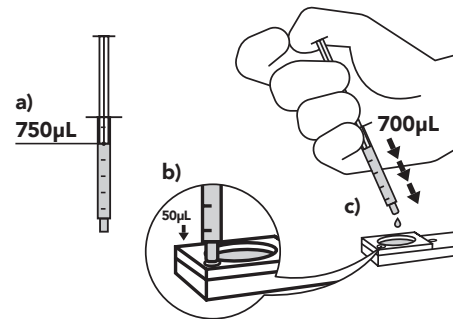
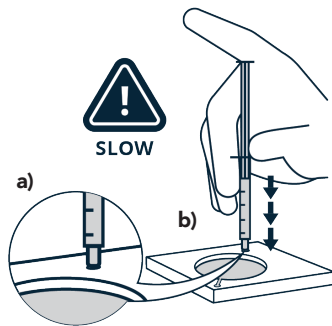
1

Equilibrate semen wash and/or IVF medium before preparing fresh semen or thawing frozen semen. Dilute semen to practical working volume of up to 900 $\mu$ L.

- For sex-sorted semen, use high-quality, non-capacitating semen wash medium with ingredients that promote motility.
- For non-sorted semen, use either high-quality semen wash medium that promotes motility or IVF medium.

2

Use 1mL syringe to draw 850 $\mu$ L aliquot of extended semen sample from Step 1.

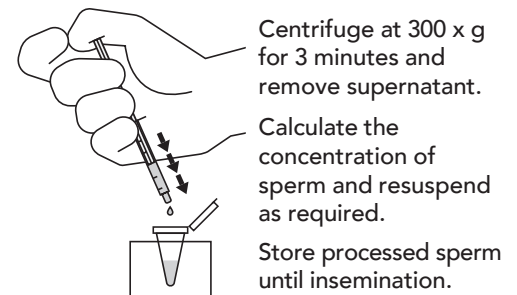
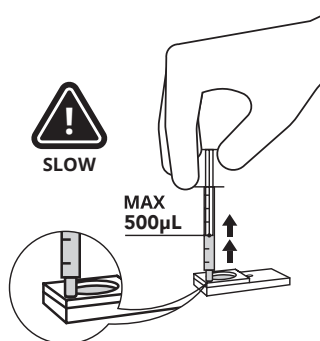
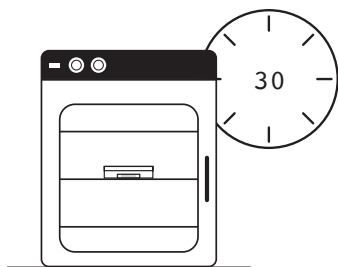


3

- Establish secure seal in the inlet port on device.
- Apply slow and steady pressure, delivering the 850 $\mu$ L sample into lower chamber through inlet channel.

4

- Use fresh 1mL semen-safe syringe to draw 750 $\mu$ L of medium. Use IVF medium or high-quality semen wash medium. For sex-sorted semen, use only high-quality, non-capacitating semen wash medium.
- Prime outlet channel with 50-100 $\mu$ L of medium.
- Dispense balance of medium onto the surface of the upper membrane.



5

Place device in Petri dish and cover. Incubate for 30 minutes. Maintain semen medium at 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 500 $\mu$ L of sperm sample from the upper chamber.

7

Either add sperm to IVF system at final concentration of  $1 \times 10^6$  sperm/mL or 2,000-25,000 sperm/oocyte.

## VetMotl® Multi (850µL) Bovine Sperm Separation Device

### VMB0850

#### – Instructions for Use –

1. Equilibrate semen wash and/or IVF medium before preparing fresh semen or thawing frozen semen.
2. For the lower device chamber: Dilute semen in semen wash medium or IVF medium to a practical working volume of up to 900µL.
  - a) For sex-sorted semen, use a high-quality, non-capacitating semen wash medium with ingredients that promote motility.
  - b) For non-sorted semen, use either a high-quality semen wash medium that promotes motility or an IVF medium.
3. Use a 1mL semen-safe syringe to draw an 850µL aliquot of the extended semen.
4. 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.
5. For the upper device chamber: Use IVF medium or high-quality semen wash medium. For sex-sorted semen, use only high-quality, non-capacitating semen wash medium.
  - a) Use a fresh 1mL semen-safe syringe to draw 750µL of medium (e.g., IVF medium or semen wash 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. Do not tilt the device to spread the medium. Gently tease out any bubbles in the medium with the syringe, being careful not to puncture the membrane.
6. Place the device in a Petri dish and cover. Incubate for 30 minutes, maintaining the semen medium at a stable working temperature and avoiding significant temperature fluctuations.
  - a) If in semen wash: A benchtop incubator in air may be used at 35–38°C.
  - b) If in IVF medium: Use your normal IVF incubator temperature (e.g., 38.5–38.7°C in 5% CO<sub>2</sub> with bicarbonate medium).
7. 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.
8. Centrifuge at 300 x g for 3 minutes and then gently remove supernatant, leaving a 100–200µL pellet. Calculate the concentration of sperm and resuspend the pellet as required.
9. Store processed sperm at appropriate pH and temperature until insemination.
10. Add sperm to the IVF system according to your protocol; for example, to a final concentration of 1x10<sup>6</sup> sperm/mL or 2,000–25,000 sperm/oocyte.