VetMotl®, Inc.

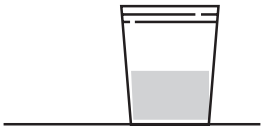
MULTI (3mL)

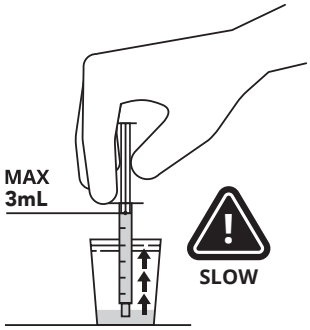
Equine Sperm Separation Device | VME3000

INSTRUCTIONS FOR USE

EQUINE LOW DOSE AI

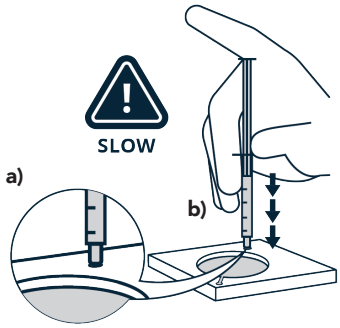
FRESH OR FROZEN-THAWED SEMEN

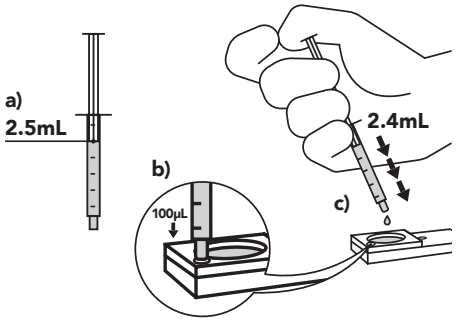




1 Fresh: Extend raw semen 1:1 (v:v) with extender at room temperature.
Frozen: Thaw frozen semen at 37°C for 30 seconds.
Extend thawed semen 1:1 (v:v) with a milk based extender.

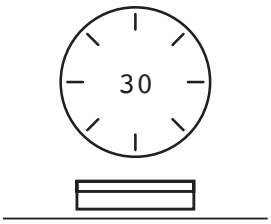
2 Use 5mL semen-safe syringe to draw 3mL aliquot of extended semen.

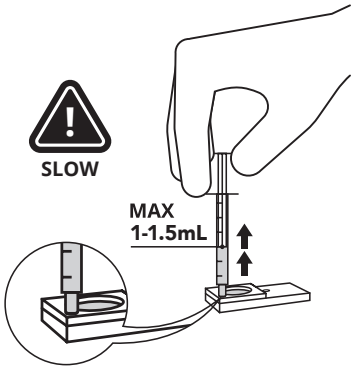


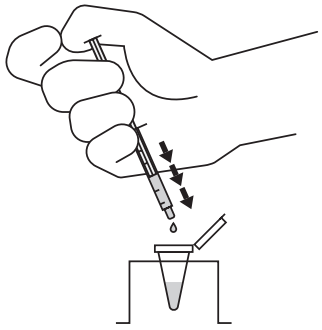


3 a) Achieve seal in the inlet port.
b) Slowly inject 3mL sample.

4 a) Draw 2.5mL of media.
b) Prime outlet channel with 100µL media.
c) Cover upper membrane with 2.4mL media.







5 Place device into a Petri dish and cover.
Incubate at room temperature for 30 minutes.

6 Insert a fresh 5mL syringe into the outlet port.
Slowly aspirate 1-1.5mL.

7 Optional: Centrifuge at 300g for 5 minutes and then remove supernatant.
Calculate the concentration of sperm and resuspend pellet as required.

VetMotl Multi (3mL) Sperm Separation Device
VME3000 Equine
Equine ART Procedure: Low-Dose AI

FRESH SEMEN

1. Extend raw semen 1:1 (v:v) extender:semen at room temperature.
2. Use 5mL semen-safe syringe to draw 3mL aliquot of extended semen. Ensure there is a minimum of 3mL of extended semen to fill the lower chamber of the device to avoid air gaps between the lower chamber and the membrane.
3. Achieve seal in the inlet port and apply slow and steady pressure to inject 3mL sample into bottom chamber via inlet port.
4. Use fresh 5mL semen-safe syringe to draw 2.5mL media. We recommend a media that is compatible with your ART system.
   a) Prime outlet port with 100µL media. We recommend an extender compatible with your ART system.
   b) Dispense the balance of the media onto the surface of the upper membrane by dropping from approximately 2cm above the membrane. Completely cover the upper membrane with media. Do not tilt the device to spread the media.
   c) Gently tease out any air bubbles in the media and avoid penetrating the membrane.
5. Place device into a Petri dish and cover. Incubate at room temperature for 30 minutes.
6. Insert a fresh 5mL syringe into the outlet port, achieving a firm connection. Very slowly aspirate 1-1.5mL of the sperm-containing fluid from the top chamber. Avoid aspirating too quickly as this can pull unwanted material from the lower chamber through the membrane into the clean sample.
7. Optional: Centrifuge at 300g for 5 minutes and then remove supernatant. Calculate the concentration of sperm and re-suspend pellet as required.

FROZEN-THAWED SEMEN

1. Thaw frozen semen (3-4 x 0.5mL straws) at 37°C for 30 seconds. Extend thawed semen 1:1 (v:v) with a milk-based extender that is compatible with your ART system.
2. Use 5mL semen-safe syringe to draw 3mL aliquot of extended semen.
3. Achieve seal in the inlet port and apply slow and steady pressure to inject 3mL sample into bottom chamber via inlet port.
4. Use fresh 5mL semen-safe syringe to draw 2.5mL media. We recommend a media that is compatible with your ART system.
   a) Prime outlet port with 100µL of media.
   b) Dispense the balance of the media onto the surface of the upper membrane by dropping from approximately 2cm above the membrane. Completely cover the upper membrane with media. Do not tilt the device to spread the media.
5. Place device into a Petri dish and cover to exclude light. Incubate at room temperature for 30 minutes.
6. Insert a fresh 1mL syringe into the outlet port, achieving a firm connection. Slowly aspirate 1mL of the sperm-containing fluid from the top chamber.
7. Optional: Centrifuge at 300g for 5 minutes and then remove supernatant. Calculate the concentration of sperm and resuspend pellet as required.