VetMotl®, Inc.

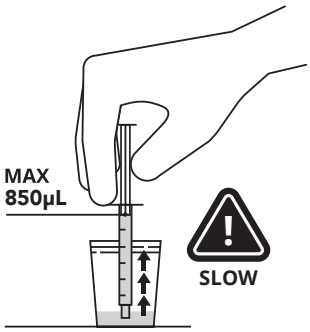
MULTI (850µL)

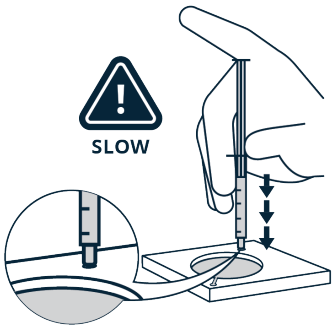
Equine Sperm Separation Device | VME0850

INSTRUCTIONS FOR USE

EQUINE ICSI & IVF

FROZEN-THAWED SEMEN





1

a) Thaw frozen semen at 37°C for 30 seconds. Warm media to 37°C.

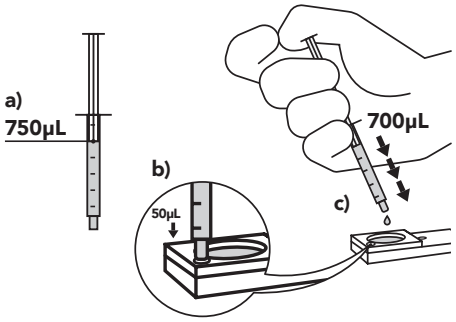
b) Place device in a Petri dish.

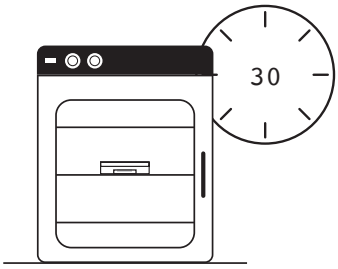
c) Use 1mL syringe to draw 850µL aliquot of warmed semen.

2

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

Achieve seal in the inlet port and apply slow and steady pressure to inject the 850µL sample into lower chamber.





3

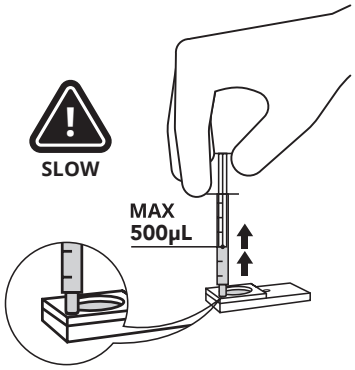
a) Draw 750µL of warmed media.

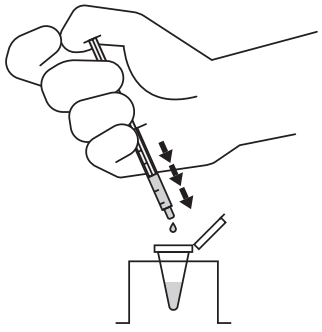
b) Prime outlet channel with 50µL of media.

c) Cover upper membrane with 700µL of media. Be careful not to touch the membrane.

4

Place cover on Petri dish and device.
Incubate at 37°C for 30 minutes in conditions appropriate for ICSI/IVF media (e.g., 5% CO_2 or benchtop).





5

Insert fresh 1mL syringe into outlet port and achieve seal. Slowly aspirate 500µL of sperm containing fluid from the top chamber.

6

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

VetMotl® Multi (850µL) Sperm Separation Device
VME0850 Equine
Equine ART Procedures: ICSI, IVF

FROZEN-THAWED SEMEN

IMPORTANT: Keep semen sample and media at 37°C throughout the entire procedure. This is extremely important; all materials must remain warm throughout the procedure.

1. Place device into a Petri dish. Thaw frozen semen at 37°C for 30 seconds.
   a) Ensure there is a minimum of 850µL of semen available to fill the lower chamber of the device. This will help to avoid any air gaps between the lower chamber and the membrane.
   b) Use a fresh 1mL semen-safe syringe to draw an 850µL aliquot of warmed semen. Ensure there are no air bubbles in the syringe.
2. Insert the syringe into the inlet port and achieve a seal. Apply slow and steady pressure to inject the 850µL semen sample into the lower chamber via the inlet port.
3. Use a fresh 1mL semen-safe syringe to draw 750µL of warmed media appropriate for ICSI/IVF procedures.
   a) Prime the outlet port with 50µL of media until a small pool forms on top of the membrane.
   b) Dispense the balance of the media (700µL) onto the surface of the upper membrane by dropping from approximately 2cm above the membrane. Completely cover the upper membrane with media. Do not touch the membrane or tilt the device to spread the media.
   c) Gently tease out any air bubbles in the media. Avoid penetrating the membrane.
4. Place the Petri dish cover on top of the Petri dish containing the device. Incubate at 37°C for 30 minutes in conditions suitable for ICSI/IVF media (e.g., 5% CO_2 or benchtop).
5. Insert a fresh 1mL syringe into the outlet port, achieving a firm connection. Very slowly aspirate 500µL of the sperm-containing fluid from the upper chamber. Avoid aspirating too quickly or with too much pressure, as this can pull unwanted material from the lower chamber through the membrane into the clean sample.

Sample is ready for use.

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

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