

## Protocol for use of SpermSafe extender with fresh semen

### Equipment required:

SpermSafe extender (prepared as below)

Milk-based extender of choice (e.g., BotuSemen Gold, EquiPlus, INRA96 or Kenneys)

Nidacon EquiPure at room temperature

Transfer pipettes

15 ml or 50 ml centrifuge tubes (e.g., Falcon tubes or similar)

Flat bottom 50 ml specimen container (e.g., Sarstedt cat# 75.9922.730)

Centrifuge set at 400 x g

Sperm counting method of choice

Insulated shipping container

### Prior to use:

1. Upon arrival, store SpermSafe in -20°C freezer
2. Prior to use, reconstitute product by adding 50 mL / 5 mL (depending on bottle size) sterile injection water or MilliQ water
  - Invert bottle until all contents are dissolved – do not shake
  - Allow ~30 min for components to be fully dissolved
  - Use at room temperature (~22°C) and keep out of direct sunlight
  - SpermSafe can be warmed briefly to 37°C to dissolve/thaw and then left to cool to ambient temperature (~22°C)
3. Use reconstituted product within 2 days and store at 4°C between use.

### Following Semen Collection:

1. Dilute semen 1:1 with a milk-based extender warmed to 33-37°C (e.g., BotuSemen Gold, EquiPlus, INRA96 or Kenneys)
  - (NB we do not recommend diluting raw semen with SpermSafe medium. SpermSafe is protein-free and cannot scavenge toxic seminal plasma proteins – milk components are required for this)
2. Isolate high-quality spermatozoa using a single layer colloid centrifugation. We recommend using EquiPure to exclude dead/dying cells, seminal plasma, and microbial contaminants.
  - a. Add 10-20 mL EquiPure at ambient temperature to a 50 mL centrifuge tube, or if smaller volumes, use a 15 mL tube and add 2 mL EquiPure.
  - b. Add an equal volume of extended semen to the tube using a Pasteur pipette or disposable syringe. Add the diluted semen slowly, making sure not to mix EquiPure and the semen. The maximum total number of sperm must not exceed  $3 \times 10^9$ .
  - c. Centrifuge at 400 x g for 20 minutes (without brake).
  - d. After centrifugation, remove the sperm pellet using a pasteur pipette and transfer to new tube.

NB SpermSafe may be used for short-term storage (<3 days) without EquiPure isolation. Centrifugation is still required to remove seminal plasma. We recommend the use of a cushion during centrifugation to tightly pellet cells and allow complete removal of seminal plasma.

3. Resuspend sperm pellet in small volume (e.g., 10 ml ) reconstituted SpermSafe™ medium at room temperature
4. Perform a sperm count, calculate total sperm number and resuspend with SpermSafe to a final sperm concentration between 20 and 50 million sperm/mL
5. Store and ship in flat-bottom specimen containers at 17°C (Please do not use syringes for storage as these are not compatible with protein-free extenders)



6. Isolated, SpermSafe-extended sperm may be stored for up to 7-14 days depending on individual stallion. Please check the motility following storage and record motility data using the form included with the shipment by submitting to our online portal:



#### DATA SUBMISSION FORM

Please use your phone to take a photo of the motility data and submit using the QR code

7. Load into semen syringe immediately before insemination, ensuring semen is not left in the syringe for more than a few minutes

For further information, training or to purchase SpermSafe or additional equipment required for this protocol, please visit our website or contact us directly via email.