# EquiPure™

### Intended Use: summary and explanation

EquiPure is designed to increase the quality and viability of equine spermatozoa by separating sperm with density centrifugation prior to cold transport, insemination, sexing and freezing or after freezing and thawing. EquiPure not only eliminates a high proportion of the abnormal spermatozoa but also removes bacteria and the source of reactive oxygen species. This significantly increases the sperm survival and their fertilizing potential.

#### Components

Silane-coated silica	EDTA
Potassium chloride	Glucose
Sodium chloride	Calcium lactate
Purified water	Sodium pyruvate
HEPES	

#### **Performance Characteristics**

pН	7.4-7.8
Osmolality (mOsm/kg H <sub>2</sub> O)	300-310

Bottles and stoppers are Mouse Embryo Assay tested

#### Storage and Stability

Store unopened bottles at 2 to  $40^{\circ}\text{C}$  and avoid temperatures above or below these values. Under these conditions

EquiPure has a shelf-life of 24 months. The expiry date is shown on both bottles and cartons.

Open and close bottles under aseptic conditions. After opening, store at 2 to 8°C when not in use. Shelf-life on the product label applies when the product is stored according to manufacturer's recommendations.

Precautions and Warnings

- When retrieving the sperm pellet, follow the instructions
  given in the pack insert to avoid inadvertent contamination
- If available, use sealed buckets during centrifugation to avoid creation of aerosols
- Clean accidental spills using a dampened cloth or paper. EquiPure causes floors and benches to be extremely slippery

- EquiPure does not represent any kind of fire or combustion hazard. A material safety data sheet is available from the distributor or manufacturer (see nidacon.com)
- Do not use any solution which shows evidence of bacterial contamination or if stopper accidently comes in contact with unsterile surfaces
- · Do not use contents if tamper-evident seal is broken
- · Does not contain antibiotics, use aseptic procedures

#### **Ordering Information Equine Products**

Description	Volume	Article No.
EquiPure	100mL	EPB-100
BotuSemen	100mL	BTS-100
BotuSemen with water	100mL	BTSW-100
BotuSemen Special	100mL	BTSS-100
BotuSemen Special with water	100mL	BTSSW-100
BotuSemen Gold	100mL	BTSG-100
BotuSemen Gold with water	100mL	BTSGW-100
BotuTurbo	100mL	BTT-100
BotuTurbo with water	100mL	BTTW-100
BotuCrio	25mL	BTC-025
SpermFilter	1pcs	BTF-001
SpermVitalStain	2x10mL	SVS-010
NidOil	100mL	NO-100
NidOil	4x100ml	NO-400K
SpermCatch (ICSI)	6 × 100 µl	SC-100
ProInsert	5 pcs	PI15-5
CryoFloater Vial	1 pcs	CFV-001
CryoFloater Straw	1 pcs	CFS-001
*For sale only in Europe		



For further technical information or assistance, please contact your distributor or the manufacturer.

Manufacturer: Nidacon, Flöjelbergsgatan 16 B, SE-431 37 Mölndal, Sweden Tel: +46-31-703 06 30, Fax: +46-31-40 54 15 E-mail: contact@nidacon.com, www.nidacon.com



NS-EPB-BK/07

## HOW TO USE EQUIPURE

# IMPORTANT NOTE:

 The procedures described below should only be performed in centrifuges with swing-out rotor. Centrifuges with fixed angle rotor should not be used.

- EquiPure does not contain antibiotics, use aseptic procedures.

### Fresh and cold semen

EquiPure can be used with fresh semen in cases where a sperm population with better motility and reduction of morphologic defects is desired. EquiPure can also be used for ejaculates from animals with low fertility rates, to improve the viability of transported cooled samples.

#### Procedure:

1. Dilute the ejaculate 1:1 with BotuSemen. Add 10-20 mL EquiPure at ambient temperature to a 50 mL centrifuge tube.

 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 semen.

Important: The maximum total number of sperm must not exceed 3 x  $10^{9}$ .

3. Centrifuge at 400 x g for 20 minutes (1600 rpm in a centrifuge with a radius of 14 cm)<sup>2</sup>, do not use the brake. If only one tube is to be centrifuged, balance the rotor with another 50 ml tube containing an equal volume of water.

4. After centrifugation, remove the ejaculate and most of the Equi-Pure using a pasteur pipette.

5. Using a new pasteur pipette retrieve the pellet and transfer to a new tube.

6. Resuspend to a concentration of 50 x 10<sup>6</sup> sperm/mL. Sperm recovery after selection with EquiPure largely depends on the initial sperm quality, but on average ranges around 40%.

# HOW TO CONCENTRATE SPERM PRIOR TO EQUIPURE

#### Spermfilter

Semen can be concentrated using SpermFilter before centrifugation with EquiPure. In this case dilute the ejaculate 1.1 with BotuSemen and filter in SpermFilter (video demo www.botupharma.com.br). Resupend in BotuSemen (10 mL). Use Equipure as described in the procedure.

### Centrifugation

Dilute the ejaculate 1:1 with BotuSemen and centrifuge at 600 x g for 10 minutes (1950 rpm in a centrifuge with a radius of 14 cm)\*, do not use the brake.

Resuspend the pellet in BotuSemen (5 -10 mL) and proceed using EquiPure as described above.

# EQUIPURE PRIOR TO FREEZING SPERM

Sperm selection by EquiPure is recommended for stallions with poor freezing tolerance. After centrifugation with EquiPure as described above, resuspend the pellet in BotuCrio to a concentration of 200x10<sup>6</sup> motile sperm/ml.

### EQUIPURE POST THAW

EquiPure can also be used with frozen/thawed samples with low viability, motility or membrane integrity. In these cases the procedure is based on selecting a sperm population with the highest viability.

After thawing, add the same volume EquiPure as the volume of the thawed straws to a 15 ml centrifuge tube. Slowly add the thawed semen sample on top of the EquiPure, ensuring there is no mixing between EquiPure and thawed semen. Centrifuge at 400 x g for 20 minutes (1600 rpm in a centrifuge with a radius of 14 cm)\*, do not use the brake.

After centrifugation, aspirate the pellet. The aspirated material should be resuspended in 4 ml BotuCrio.

## \*To achieve the correct g force:

Rpm =  $\sqrt{[(q/(1.118 \times r)] \times 10^3]}$ 

 ${\sf r}$  = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position

For example; to achieve 400 x g when radius = 140 mm the centrifuge speed must be:

Rpm =  $\sqrt{[(400/(1.118 \times 140)] \times 10^3 = 1597]}$ 

