



# Equine Product Manual

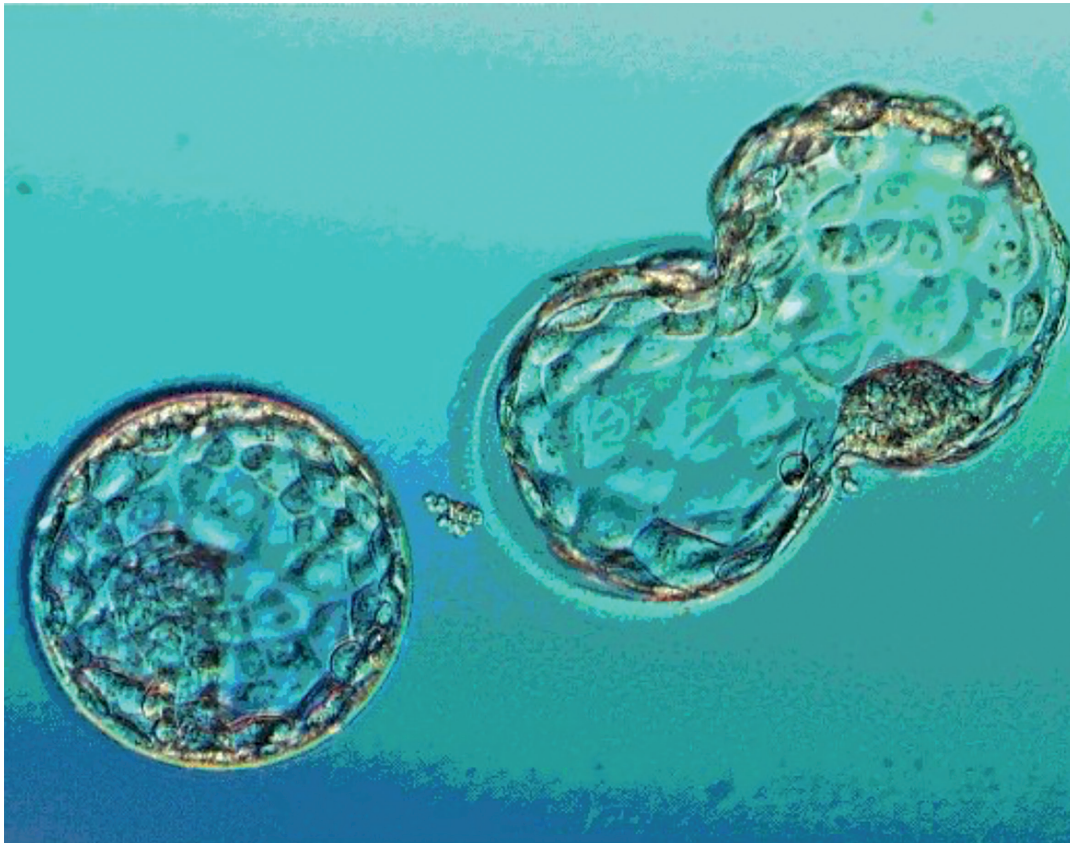


## Nidation

1. The building of a nidus, a nest, as with birds.
2. Implantation of the fertilized ovum (zygote) and the building of a nest in the endometrium, the placenta.

## Conception

1. The union of male and female gametes, the sperm and egg.
2. An impression or idea.



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### Introduction

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Nidacon considers many different factors when designing its products. We hope that the attention to detail has helped to create products which will lead to better results. We aim to work in close relation with our customers; they are the cornerstones of our research department.

We take pride in the development of our products and make sure we respond to the needs of our customers and research colleagues. All our products are developed in close cooperation with professionals in the different fields.

In the early 2000s Nidacon realized that Equine sperm should get the same professional treatment as human sperm and developed EquiPure, based on PureSperm, the equivalent for human sperm.

In collaboration with BotuPharma, Brazil, we also provide our equine customers with extenders and much more.

#### Corporate Social Responsibility - CSR

*We shall contribute to a sustainable world by taking responsibility for human welfare and our impact on the environment and society as a whole.*

In ecology, sustainability is the capacity to endure; it is how biological systems remain diverse and productive indefinitely. In more general terms, sustainability is the endurance of systems and processes. The principle for sustainability is sustainable development, which includes the four interconnected domains: ecology, economics, politics and culture.

Nidacon believes in the importance of our legacy and works actively to make a positive imprint and to take responsibility for our impact on our world. A more conscious use of resources is essential in order to maintain healthy ecosystems and environments. Having a well-functioning system for waste recycling was therefore a natural step for us.

We investigated our express deliveries and came to the conclusion that we should compensate for carbon emissions. Therefore from now on, we pay an extra fee for every package that is shipped from us. UPS has a program called "UPS Carbon Neutral" which we have joined and, for all other deliveries, we compensate by planting trees through WWF.

We are also a member of an organization called CSR West Sweden which focuses on global sustainability. To ensure our continual development we will keep attending seminars, courses and conferences with this focus.

We are very proud to also being a part of the research at the Leibniz Institute for Wildlife research in Berlin Germany, in the quest to prevent the extinction of several animal species (the white rhinoceros for example).



## Shelf life • Packaging

### Shelf life

EquiPure is stable for two years at room temperature. After opening the products should be stored at 2 to 8°C when not in use.

The extenders are stable for two years in powder form. After reconstitution in water they can be in refrigerator for 3 days. It is also possible to freeze

reconstituted extender and store for up to a year. BotuCrio should be used within 3 days after thaw. It is possible to refreeze, but there is then a risk of build of fat from the egg yolk which is seen as black debris in the microscope. This is however harmless to the spermatozoa.

### Packaging

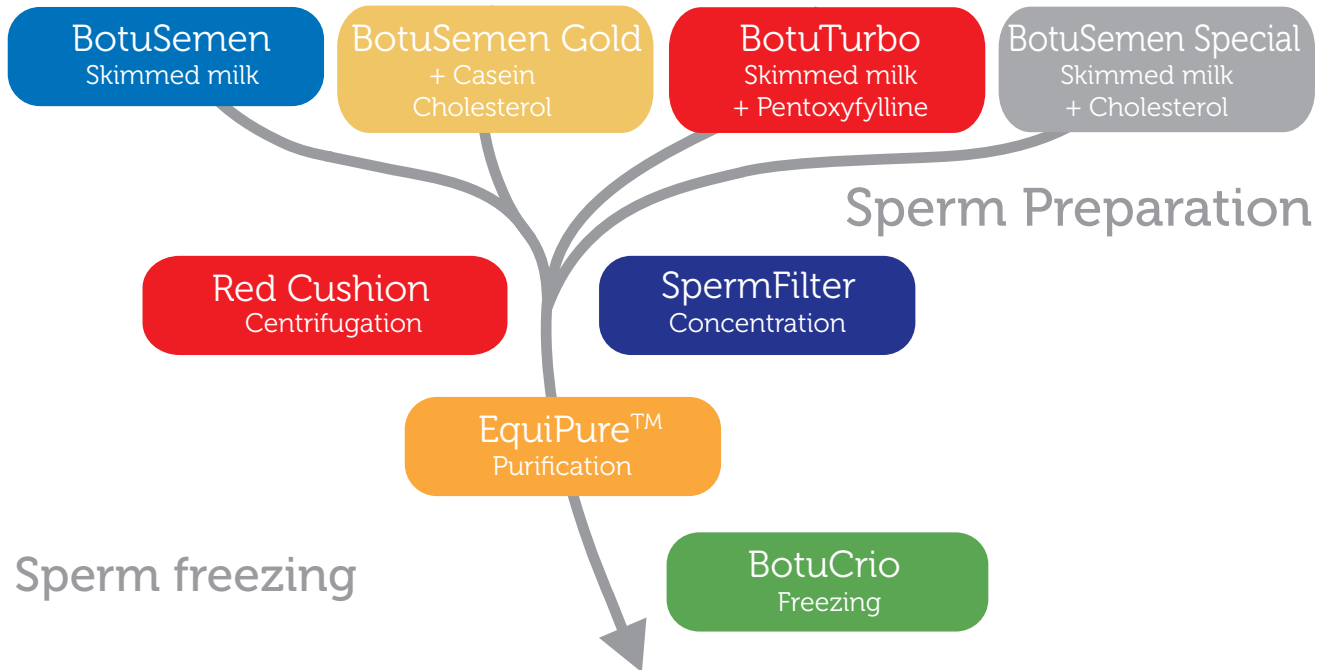
The packaging for Nidacon's products has received the same care and attention to detail as the

design of the products. All packaging are MEA-tested to ensure lack of toxicity.



## Nidacon Equine Product Range

### Semen extenders



### Sperm freezing

### ICSI

### Embryo transportation

### Diagnostics

#### Ordering information

Description	Volumen	Art. no.
EquiPure	EPB-100	100 mL
BotuSemen	BTS-100	100 mL
BotuSemen with water	BTSW-100	100 mL
BotuSemen Special	BTSS-100	100 mL
BotuSemen Special with water	BTSSW-100	100 mL
BotuSemen Gold	BTSG-100	100 mL
BotuSemen Gold with water	BTSGW-100	100 mL
BotuTurbo	BTT-100	100 mL
BotuTurbo with water	BTTW-100	100 mL

Description	Volumen	Art. no.
BotuCrio	BTC-025	25 mL
SpermFilter	BTF-001	1 pcs
RedCushion	RC-100	100 mL
SpermVitalStain	SVS-010	2x10 mL
NidOil	NO-100	100 mL
NidOil	NO-400K	4x100 mL
BotuEmbryo	BE-008	8 mL
SpermCatch	SC-100	6 × 100 µL
ProInsert	PI15-5	5 pcs
CryoFloater Vial	CFV-001	1 pcs
CryoFloater Straw	CFS-001	1 pcs



### EquiPure™

Ready-to-use density gradient solution, optimized for the preparation of density gradients for the separation and purification of equine sperm.

Silane coated, silica colloid in an isotonic solution.

Shelf-life: Two years at room temperature.



### BotuSemen

Extender based on skimmed milk to transport cooled semen, to preserve at 5 or 15°C and for centrifugation pre-freeze. Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Suitable for most stallions.

Shelf-life: Two years in powder form, three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water



### BotuSemen Special

Extender based on skimmed milk with the addition of cholesterol.

BotuSemen Special is highly recommended for pre-freezing centrifugation and is suitable for spermatozoa with sensitivity to cooled transport.

Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Shelf-life: Two years in powder form, three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water.



### BotuTurbo

Extender based on skimmed milk with addition of Pentoxifylline.

Ideal for fresh insemination or cooled semen transport at 5°C.

Suitable for stallions with poor sperm motility.

Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Shelf-life: Two years in powder form, three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water.

## Products



### BotuSemen Gold

Extender with casein and cholesterol. Suitable for all stallions, especially for those with suspected premature sperm capacitation, and with sensitivity to cooled transport.

Contains Penicillin K 1 g/l and Gentamicine 1 g/l.

Shelf-life: Two years in powder form, stored at 2-8°C. Three days after reconstitution in water at 2-8°C, one year frozen after re-constitution in water..



### BotuCRIO

Freezing extender with egg-yolk. BotuCrio contains a low concentration of glycerol, which in combination with one of several amides, results in better sperm integrity, sperm viability, better post-thaw total and progressive motility, as well as sperm velocity, compared to other commercially available freezing extenders.

Contains Gentamicine 0.5 g/l.

Shelf-life: 1 year frozen. Three days at 2-8°C after thaw.



### RedCushion

Cushion fluid of high density used for semen centrifugation. It reduces damage from centrifugation through sperm damping over the RedCushion on the bottom of the tube. Because it has a reddish coloration, it allows for easy visualization of the sperm layer and its removal.

Shelf-life: 2 years at 2-8°C.



### SpermFilter

Hydrophilic synthetic membrane which efficiently concentrates stallion sperm.

Shelf-life: Re-usable, stored at room temperature.





### SpermCatch™

For slowing down sperm prior to ICSI without using polyvinylpyrrolidone (PVP). To avoid ICSI injection of PVP, it contains only natural products for increasing the viscosity.

Shelf-life: 1 year at room temperature



### NidOil™

Sterile, light paraffin oil for use as an overlay during gamete, zygote and pre-embryo culture in the incubator, or during mani-pulations outside the incubator. No additives, UV-protective packaging.

Shelf-life: 2 years at room temperature



### BotuEmbryo

BotuEmbryo is a media for maintenance and transport of embryos. Its packaging does not require the use of syringes and needles. It has an innovative formulation based on scientific studies carried out by national and international researchers.

Transport temperature: Ambient for up to 3 days.

Storage temperature: 1 year at 2-8°C



### Sperm VitalStain™

One step staining technique for assessment of equine sperm vitality and morphology, a basic tool for semen analysis.

Shelf-life: 2 years at 10-40°C.

# EquiPure – density centrifugation

## Background

A normal semen sample (ejaculate) is made up of seminal fluid which contains a number of different cells, cell debris, microbiological and biological substances.

The different cell types contained in semen are normal motile sperm, juvenile sperm, senescent sperm (no fertilisation function) and sperm with DNA breaks. Epithelial cells, immune cells and cell

debris (detritus) are also present in the semen, as are bacteria and possibly viruses.

Moreover, the seminal fluid contains biologicals such as sperm decapacitating factors and reactive oxygen species (ROS), both of which negatively affect fertilisation.

EquiPure removes all these detrimental agents and increases fertilization and pregnancy rates.



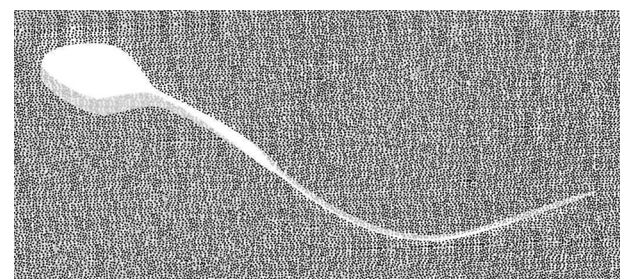
## What is EquiPure and how does it work?

### EquiPure decontaminates and purifies the sperm.

EquiPure contains colloidal silica which has gone through the process of silanization. A colloid is a dispersion of particles that are small enough to not be affected by the force of gravity ( $\leq 1 \mu\text{m}$ ) but large enough ( $> 1 \text{nm}$ ) to not be defined as a true solution. It is this which gives EquiPure its specific functional characteristic.

Silica occurs naturally in crystalline form and it is the most abundant component of the earth's crust. It is also known to play an important role in many biological systems and in nature.

However, the silica present in EquiPure is manufactured through an industrial process which ensures it meets our requirements and specifications. This synthetic colloidal silica contains amorphous silicon dioxide ( $\text{SiO}_2$ ). The spherical silica particles have a smooth surface and are dispersed in a fluid which is referred to as a silica sol. The fluid in this case is water. Furthermore, it is a stable dispersion. This means that the solid particles do not settle or agglomerate at any significant rate.



*Sperm cell and colloid: a ratio of approximately 1000:1.*

The colloidal solution is then adjusted with different salts in order to have the right physiological composition and osmolality for equine spermatozoa, pH and osmotic values are set to lie intermediately between those in the semen

EquiPure is of a higher density than immotile/dead sperm, debris, epithelial cells, leukocytes, bacteria and sperm with DNA-breaks.

EquiPure is of a lower density than motile, intact sperm. Therefore it separates these agents at centrifugation.

## EquiPure™

### Important:

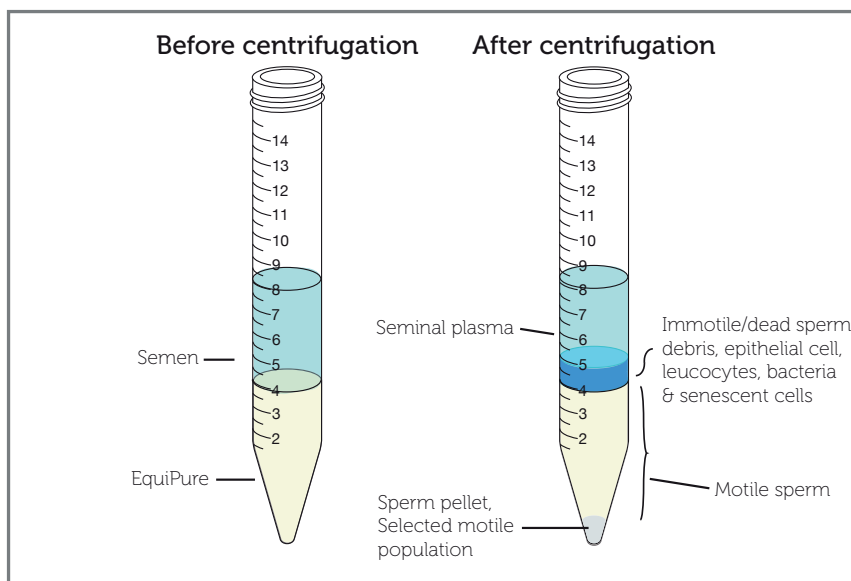
- The procedures described below should only be performed in centrifuges with swing-out rotor. Centrifuges with fixed angle rotor should not be used.
- EquiPure does not contain antibiotics, use aseptic procedures.
- The protocol states g-force and not RPM. It is important to have the correct g-force and we advise you to use the equation to make sure it's

the recommended g-force for the centrifugation. Different sizes of centrifuges requires different RPM to achieve the same g-force.

To achieve the correct g force:

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

A calculator can also be found on this QR-code.



## General care and use

- All solutions should be brought up to room temperature before use to avoid the temperature fluctuations which are detrimental to sperm survival.
- Open and reseal bottles in a laminar air-flow bench (if possible) using sterile techniques to avoid contamination.
- Store all opened bottles at 2-8°C after re-sealing.
- Under these conditions the same shelf-life applies even after opening.

## Tips

- When retrieving the pellet after the gradient centrifugation, care must be taken to avoid contaminating the pellet with components of the ejaculate. Therefore we recommend using a new pipette after removing most of the gradient to avoid contamination, for example, by bacteria.

# EquiPure – density centrifugation

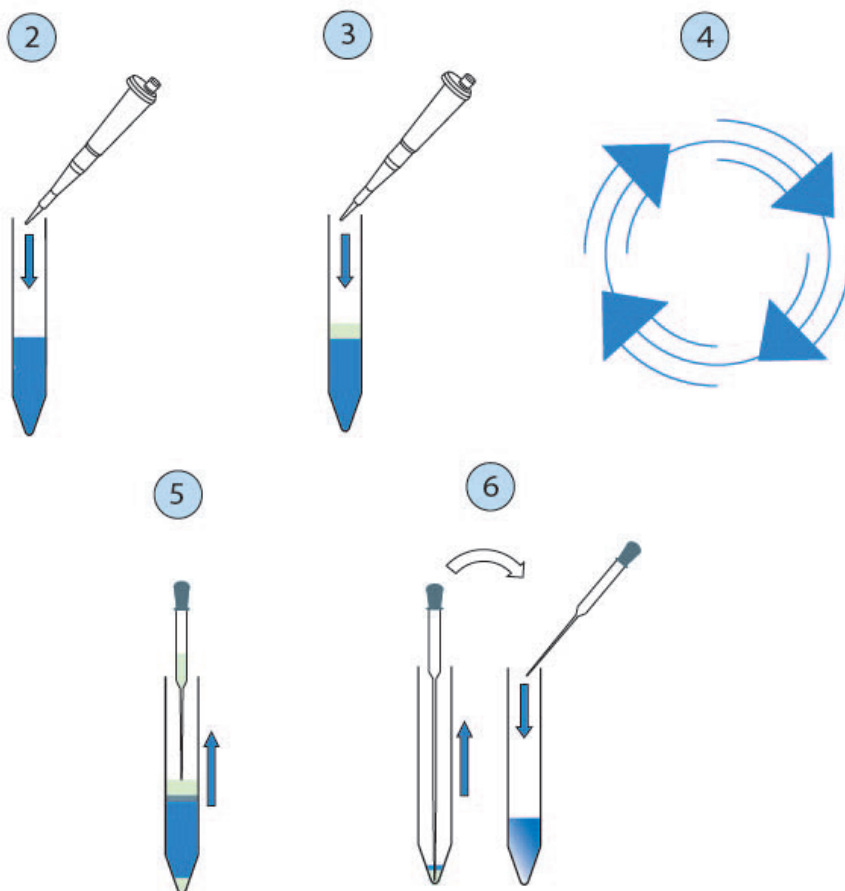
## Reagents and equipment

- Semen extender - we recommend BotuSemen
- 50 or 15 mL conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor.

## Procedure

1. Initially dilute the ejaculate 1:1 with BotuSemen, BotuSemen Special or Botu Semen Gold.
2. 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.
3. 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. Important: The maximum total number of sperm must not exceed  $3 \times 10^9$ .
4. Centrifuge at  $400 \times g$  for 20 minutes (1600 rpm in a centrifuge with a radius of 140 mm), do not use the brake. If only one tube is to be centrifuged, balance the rotor with another 50 mL or 15 mL tube containing an equal volume of water.
5. After centrifugation, remove the ejaculate and the most part of the EquiPure using a pasteur pipette.
6. Using a new pasteur pipette, retrieve the pellet and resuspend in BotuSemen to a concentration of  $50 \times 10^6$  sperm/mL.

*Sperm recovery after selection with EquiPure largely depends on the initial sperm quality, but on average ranges around 40%.*



## Concentrating sperm prior to EquiPure treatment

### 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](http://www.botupharma.com.br)). Then resuspend in BotuSemen (10 mL). Use Equipure as described above.

### 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 140 mm), 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  $200 \times 10^6$  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 of the 500 µl-straws, 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 the EquiPure and thawed semen.

Centrifuge at 400 x g for 20 minutes (1600 rpm in a centrifuge with a radius of 140 mm), do not use the brake. After centrifugation, aspirate the pellet.

The aspirated material should be resuspended in 4 mL BotuCrio.

Insemination is performed preferably at the end of the uterine horn on the site of ovulation.



## Extenders

### Background

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The semen extenders from BotuPharma come in powder form. The advantage is that the extenders stay stable for three years. As is well known, antibiotics in solution break down and lose their efficiency. Penicillin especially is not stable in solution for more than a week or two.

Also, the extenders can be stored in room temperature until constituted in water.

Nidacon provides water with the powdered extenders. It is vital not to use any kind of water. It needs to be sterile and completely de-ionized not to disturb the composition of the extender.

All extenders contain Penicillin K 1 g/l and Gentamicin 1 g/l.

All extenders can be stored at 2-8°C for up to three days after reconstitution in water.

Already reconstituted extender can be frozen and stored frozen for up to one year.

Presentation:

5 packets (5 x 8.5 g) including funnel for easier reconstitution in water.

The extenders are provided with or without PureWater. It is important to use a sterile water with zero osmolality to maintain the balance in the extenders.

### BotuSemen

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BotuSemen is an extender that was developed after years of research and its current formulation allows the safe transport of sperm until the time of insemination. It is also used to dilute the ejaculate during the freeze-spinning process.

It can also be used to extend semen prior to using EquiPure.

Composition:

Skim milk powder UHT sterilized, sugars, amino acids, antioxidants and excipients.

Stability:

2 years in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

Preparation:

Add all content of the packet in 100 mL PureWater and shake very well.

Usage:

For transport of cooled semen, dilute to concentration of 30 to 50 million sperm/mL.

Extend 1:1 (v/v) and wait 15 minutes for insemination with non cooled semen.

### BotuSemen Special

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BotuSemen Special is an extender especially developed for stallions with semen sensitive to the cooling process. Its composition is based on addition of essential lipids to maintain the stability of the sperm plasma membrane.

It can also be used to extend semen prior to using EquiPure.

Composition:

Skim milk powder UHT sterilized, sugars, amino acids, antioxidants, cholesterol and excipients.

Stability:

2 years in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

Preparation:

Add all content of the packet in 100mL PureWater and shake very well.

Usage:

For transport of cooled semen dilute to concentration of 30-50 million sperm/mL.

Extend 1:1 (v:v), wait 15 minutes for insemination with non cooled semen.

## BotuTurbo

A density gradient will effectively remove lymphocytes, BotuTurbo is an extender developed to improve the vigor of slow moving sperm. It is highly recommended for insemination and transport of fresh semen from stallions that need more movement.

**Composition:**

Skim milk powder UHT sterilized, sugars, amino acids, antioxidants, pentoxifylline and excipients.

**Stability:**

2 years at room temperature in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

**Preparation:**

Add all content of the packet in 100 mL PureWater and shake very well.

**Usage:**

For transport of cooled semen dilute to concentration of 30-50 million sperm/mL.

Extend 1:1 (v:v), wait 15 minutes for insemination with non cooled spemen.

Highly recommended for reinforcement breeding (thoroughbred).

## BotuSemen GOLD

BotuSemen Gold is based on casein which in high concentrations contributes to a delay in sperm capacitation process, improving pregnancy rates.

It is can also be used to extend semen prior to using EquiPure.

Milk casein phosphoproteins protects sperm cells from seminal plasma proteins (HSP-1, 2) which occur in the seminal plasma.

**Composition:**

Casein, sugars, amino acids, antioxidants, cholesterol and excipients.

**Stability:**

2 years in refrigerator in dry form, 1 year reconstituted and frozen, three days in refrigerator after reconstitution in water.

**Preparation:**

Add all content of the packet in 100 mL PureWater and shake very well.

After dilution wait 15 minutes befor use.

**Usage:**

In cases where centrifugation before cooling is necessary, resuspend to at least  $100 \times 10^6$  spetz/mL. Use dilution table below:

Spermatic concentration	Dilution (v:v)
< $90 \times 10^6$ /mL	1:1 Centrifuge and resuspend to $100 \times 10^6$ spetz/mL
90 – $120 \times 10^6$ /mL	2:1
130 – $160 \times 10^6$ /mL	3:1
170 – $210 \times 10^6$ /mL	4:1
220 – $250 \times 10^6$ /mL	5:1
260 – $300 \times 10^6$ /mL	6:1

# Freezing extender

## BotuCrio®

BotuCrio is the result of years of research and fertility trials at the state university of Sao Paolo, Brazil and is extensively tested in the field.

The extender contains the optimal components to protect cells from the damage caused by freezing and to protect their fertility after thawing.

BotuCrio contains a low concentration of glycerol, which in combination one of several amides, results in better sperm integrity, sperm viability, better post-thaw total and progressive motility, as well as sperm velocity, compared to other commercially available extenders.

Studies have indicated that stallion sperm frozen in BotuCrio are more fertile compared with other extenders.

These in vitro results have been confirmed in field experiments which show higher pregnancy rates after insemination.

The higher pregnancy rates achieved with semen frozen in BotuCrio results in a higher pregnancy rate per cycle and less inseminations per pregnancy. This reduces the cost to breed a mare.

BotuCrio is today used for both good and poor freezing stallions in freezing centers in Australia, The Middle East, North and South America as well as in Europe.

Composition:

Sugars, antioxidants, amino acids, egg yolk, cryoprotectants and excipients and Gentamicine 0.5 g/l.

Stability:

1 year frozen. Three days in refrigerator after thaw.

Use:

BotuCrio is shipped and stored frozen. Thaw BotuCrio in 37°C water until completely thawed. Let rest on bench 5 minutes. Ensure that it is well in room temperature before use. The fat from the egg yolk must have time to completely dissolve, it can otherwise be seen as black debris. This however is completely harmless to the spermatozoa.

After semen collection:

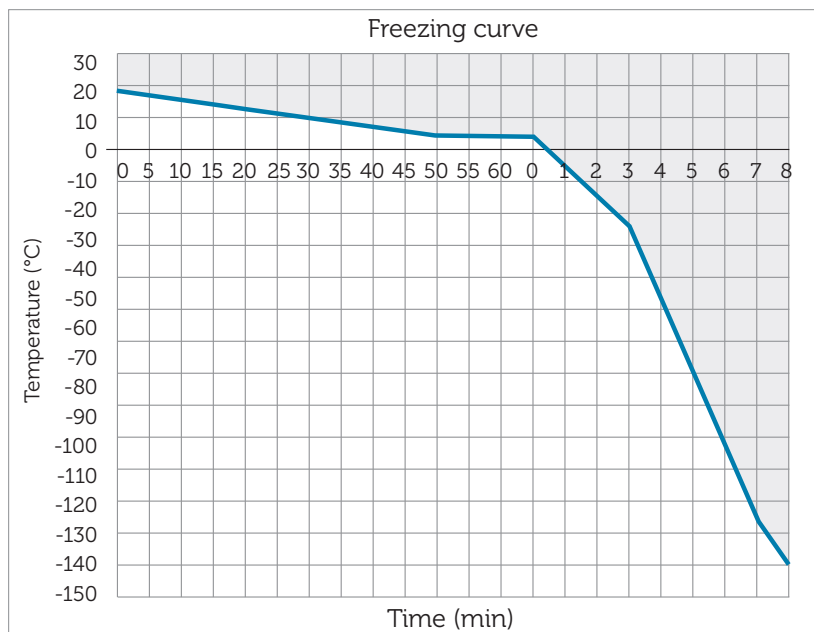
1. Determine sperm parameters: volume, motility and concentration.
2. Dilute the ejaculate with extender 1:1 (v:v). Use BotuSemen Gold for optimal results
3. Centrifuge at 600 x g for 10 minutes or with Red-Cushion at 1000 x g for 20 minutes.
4. Remove supernatant.
5. Resuspend pellet with BotuCrio to 200x10<sup>6</sup> or 100x10<sup>6</sup> sperm/straw.
6. Pack and seal straws.
7. Leave straws in refrigerator at 5°C for 20-30 minutes.
8. Add 6 cm (2.5 inches) of nitrogen into styrofoam box.
9. Put straws 3-6 cm (1.2-2.4 inches) above the liquid nitrogen surface for 20 minutes.
10. Plunge the straws directly into the liquid nitrogen.

Freezing machine:

1. Set machine to 5°C.
2. Add straws to machine and cool at 5°C for 20-30 minutes.
3. Start the cooling rate to -30-60°C/minute until at least -120°C.
4. Plunge straws directly into the liquid nitrogen.

Thaw straws in water batch at 37°C for 30 sec or at 46°C for 20 sec.

Using EquiPure pre or post thaw will increase survival and fertilization rates.





## Red Cushion

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A cushion fluid of high density used for semen centrifugation. It reduces damage from centrifugation through sperm damping over the RedCushion on the bottom of the tube. It produces less compacted pellets with high sperm recovery rate due to the possibility of working with intensity and time of centrifugation above those used in conventional protocols. Because it has a reddish coloration, it allows easy visualization of the spermatozoa layer and its removal.

Two different method can be used:

Usage (1):

1. Aspirate 3 mL of RedCushion with syringe and needle, and deposit at the bottom of the centrifugation tube.
2. Dilute the ejaculate with BotuSemen in the proportion of 1:1 v/v.
3. Incline the tube to 45° and slowly deposit the diluted semen on the wall with plastic Pasteur pipettes so that no mixing occurs between the semen and the RedCushion.
4. Centrifuge at 1000 x g for 20 minutes.
5. With help of a pipette remove the sperm layer deposited on top of RedCushion.
6. Resuspend the sperm with BotuSemen or BotuCrio, according to the procedure to be held.

Usage (2):

1. Dilute the ejaculate with BotuSemen in the proportion of 1:1 v/v.
2. Insert the diluted semen into a centrifuge tube.
3. With help of new syringe and needles deposit 3 mL of RedCushion under the diluted semen.
4. Centrifuge at 1000 x g for 20 minutes.
5. Remove the supernatant.
6. Aspirate the formed layer over the RedCushion.
7. Resuspend the sperm with BotuSemen or BotuCrio, according to the following procedure.

Storage:

Store at 2-8°C. Shipped at room temperature.



## SpermFilter

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A more sensitive way of concentrating semen is Sperm Filter which is a synthetic membrane with a porosity that allows the passage of seminal plasma and bacteria, retaining only sperms. The filter eliminates the need of a centrifuge, being less harmful to sperm when compared to the centrifugation usually used to concentrate sperm.

Usage:

1. Carefully remove the filter from the package. Place the Petri dish on a surface heated to a temperature of 30-35°C.
2. Dilute the semen with BotuSemen and pour it into the SpermFilter.
3. To concentrate the sperm, gently swirl the diluted semen around the filter and repeatedly touch the bottom of filter on the bottom of Petri dish until only a slight amount of liquid is evident on the filter membrane.

4. Wash the filter with appropriate volume of either BotuSemen or BotuCrio extender. Repeat swirling motions to resuspend the sperm and pour it into a beaker. If sperm recovery is low due to smaller size of the sperm, it may be necessary to refilter the ejaculate.

The SpermFilter can be reused for the same stallion at least 10 times. Wash it with sterile distilled water and dry at 37°C.



## SpermCatch™

SpermCatch is a viscous solution designed to modulate sperm motility in a physiological manner. The preparation is supplied ready for use in a sterile isotonic salt solution.

SpermCatch is an alternative to PVP (polyvinylpyrrolidone) which today is the most common substance used for slowing down sperm prior to ICSI. However, PVP has been reported to cause problems, such as damaging the sperm plasma membrane. It may also interfere with sperm nucleus condensation.

SpermCatch is a solution without PVP, instead it contains hyaluronic acid which is a natural component.

The sperm is injected into the oocyte, hyaluronic acid is preferable to plastic (PVP).

Components:

Salts, Bicarbonate, Sugars, EDTA, HEPES, Human Serum Albumin, Hyaluronic Acid.

Stability:

1 year at room temperature.

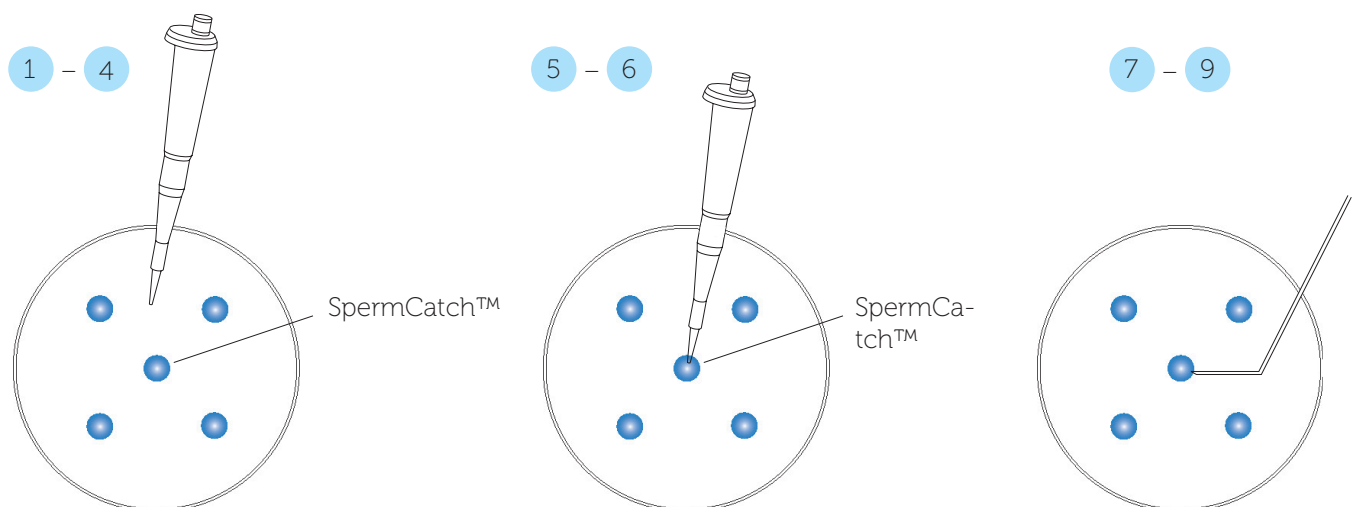
## Reagents and equipment

SpermCatch™  
NidOil™  
Injection media

Sterile pipettes  
ICSI equipment  
Petri dish

## Procedure

- Place a 10 µL drop of SpermCatch in the middle of a petri dish.
- Place 4 drops of 10 µL injection media around the SpermCatch drop in the petri dish.
- Immediately cover the drops with NidOil.
- Incubate for 30 minutes in CO<sub>2</sub> environment at 37°C.
- Add 1 µL of prepared sperm suspension to the middle of the SpermCatch drop.
- Incubate for 10 minutes in CO<sub>2</sub> environment at 37°C.
- Fill your injection pipette with SpermCatch to avoid the sperm sticking to the inside of your pipette. It will also help you to make a controlled injection.
- Immobilise the individual sperm by using the injection pipette to "nick" the sperm tail.
- Aspirate the immobilised sperm.
- Move to one of the oocyte droplets. Focus on the oocyte and position the oocyte with the holding pipette. Bring down your injection pipette and inject the sperm. Make sure that the oolemma is broken before you expel the sperm.



## NidOil™

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NidOil is a paraffin oil which has been specifically chosen and then treated in our production facilities to ensure that its purity and handling characteristics are suitable for using as an overlay when culturing gametes and embryos.

NidOil does not require washing before use, and it is neither too sticky nor too viscous, to facilitate pipetting.

Many questions have been raised lately to whether the oil that is used for covering an embryo culture can actually damage the embryo. All oil batches today from different manufactures are tested for sterility, endotoxins and a mouse embryo assay showing blastocyst development. This is apparently not enough, since damage to cultures has been ob-

served with an approved batch of oil. One answer could be peroxidation of the oil which has been investigated in several publications and found to be harmful to fertilisation and embryo development when over a certain level.

Our stringent quality assurance control helps maintain our standard for low endotoxin and peroxide levels and also ensures our products are free from microbiological contamination. There have been several reports of paraffin oils becoming embryo-toxic after exposure to light on the laboratory bench. As a precaution against any possible light-induced changes, NidOil is packaged in amber, screw-top bottles.

## Recommendations before use

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NidOil should be equilibrated in the same way as the culture medium before use to avoid differences in

temperature and gaseous content between the components of the culture system.



## Embryo transport media

### BotuEmbryo

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BotuEmbryo is a media for maintenance and transport of embryos. Its packaging does not require the use of syringes and needles. It has an innovative formulation based on scientific studies carried out by national and international researchers.

#### Usage:

Transfer embryo to a Petri dish containing BotuEmbryo.

#### Storage and stability:

Transport temperature: Ambient for up to 3 days.

Storage temperature: 1 year at 2-8°C

BotuEmbryo is highly stable, allowing the embryo transport at ambient or refrigerated temperature in appropriate containers for up to 24 hours.

Performance pH: 7.0-7.4

Osmolality: 290-310

### Components

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Amino acids, vitamins, sugar, phenol red, hepes, streptomycine amphotericin, bovine serum albumin and preservatives.



## Sperm VitalStain™

Sperm VitalStain is an optimised staining technique for assessment of Equine sperm vitality and morphology, which are two of the basic tools used in semen analysis.

The Sperm VitalStain technique for vitality assessment is based on the principle that dead cells (i.e. those with damaged plasma membrane) will take up the eosin and stain red. Nigrosine is used as a counterstain to facilitate visualization of the unstained (white) live cells.

## Technical Information

Sperm VitalStain is a salt solution containing the historical colouring agents eosin and nigrosine, the eosin being taken up by dead sperm through porous

Sperm VitalStain can also be used to highlight details when assessing normal and abnormal sperm (morphological examination).

Sperm VitalStain can be stored at room temperature for up to 2 years from production date, ensuring minimal wastage. It should not be stored in refrigerator.

membranes, and the nigrosine providing a dark contrast background to emphasise the unstained cells.

## Reagents and equipment

Light microscope (40 – 100 x magnification)

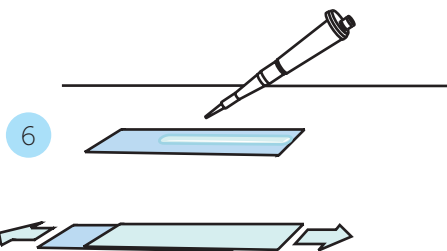
Microscope slides

## Procedure

1. Shake the bottle of Sperm VitalStain before use.
2. Take an equal amount of Sperm VitalStain and the sperm sample (eg. 50  $\mu$ L SVS + 50  $\mu$ L sample). Use for example an eppendorf tube.
3. Mix well.
4. Leave for 30 seconds at room temperature.
5. Prepare a slide using your conventional method or use the method recommended by Nidacon.
6. Transfer a 20  $\mu$ L drop onto a labelled microscope slide with a pipette, making a string/line of fluid in the middle of the slide.
7. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
8. Air dry the two slides and examine. If you want to store for later use, mount the slides with DPX or equivalent mountant, and a cover slide.
9. Examine using a bright field 40 x objective or a 100 x objective under oil immersion.
10. Count 200 sperm, the white (unstained) are classified as alive and the red or pink are classified as dead. Sperm coloured only at the neck region are classified as alive.

## Tips

- The 100x objective with immersion oil will give you a very clear picture of stained versus unstained sperm.



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