

BoviPure™ System

Intended Use

BoviPure™ System is designed to increase the quality and viability of bovine spermatozoa by separating sperm with density centrifugation prior to cold transport, insemination, sexing and freezing or after freezing and thawing. BoviPure™ 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	Magnesium Sulphate
Potassium chloride	Purified water
Calcium chloride	Pyruvate
Sodium bicarbonate	HEPES
Potassium dihydrogen ph.	EDTA
Sodium chloride	Glucose
Sodium Citrate	BSA
Calcium Lactate	

Performance Characteristics

pH	7.4-8.0
Osmolality (mOsm/kg H ₂ O)	290-310
Endotoxin transfer during treatment	<1.0 EU/mL
Human sperm survival 18 hours after density gradient separation	>70%

Bottles and stoppers are M.E.A. tested

Storage and Stability

Store unopened bottles of BoviPure™ and BoviDilute™ at 2 to 40°C and avoid temperatures above or below these values. Under these conditions they have a shelf-life of 24 months.

Store unopened bottles of BoviWash™ at 2 to 30°C. Under these conditions it has a shelf life of 12 months. The expiry date is shown on both bottles and cartons.

Open and close all 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 and handled according to manufacturer's recommendations.

No antibiotics, unstable additives or preservatives have been added by the manufacturer.

Precautions and Warnings

- When retrieving the sperm pellet, follow the instructions given in this pack insert to avoid inadvertent contamination
- Use aseptic procedures at all times
- If available, use sealed buckets during centrifugation to avoid creation of aerosols
- Clean accidental spills using a dampened cloth or paper. BoviPure™ causes floors and benches to be extremely slippery
- BoviPure™ System 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 accidentally comes in contact with unsterile surfaces
- Do not re-use
- Do not use contents if tamper-evident seal is broken
- Not for drug, household or other uses. Avoid ingestion and contact with eyes.
- Please check for regulatory compliance governing the use of ART products in your country

Ordering Information BoviPure™ System

Description	Volume	Article No.
BoviPure™	100mL	BP-100
BoviDilute™	100mL	BD-100
BoviWash™	100mL	BW-100



www.nidacon.com

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



BoviPure Single Layer Fresh Semen

MATERIALS REQUIRED

- BoviPure™
- BoviDilute™
- BoviWash™
- Conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor

HOW TO USE A SINGLE LAYER BOVIPURE FOR FRESH SEMEN

Important Notes:

- The procedures described below should only be performed in centrifuges with swing-out rotor. Centrifuges with fixed angle rotor should not be used.
- BoviPure System **does not contain antibiotics**, use aseptic procedures*.

- Prepare the appropriate amount of solutions according to the table below.
- Bring all materials to room temperature.
- Extend the ejaculate with BoviWash™ 1:1 (max conc 250 million/mL).

1. Using a sterile pipette, transfer 80% diluted BoviPure™ to a conical centrifuge tube (see volumes and sizes above).
2. Using a new sterile pipette, layer extended ejaculate on top of the BoviPure™ layer.
3. Centrifuge at 300 x g for 25-30 minutes** at room temperature, do not use the brake. (longer time for larger volumes).
4. Use a sterile Pasteur pipette and aspirate, in a circular movement from the surface, everything except the pellet and 4-6 mm of the BoviPure™ Layer.
5. Using a new sterile pipette, transfer and resuspend sperm pellet in 1 mL BoviWash™ in a new sterile tube (10-15 mL).
6. Dilute to desired sperm concentration with BoviWash™.

Note: we recommend preparing two BoviPure™ single layer preparations for each sample, to reduce the risk of overloading a single gradient and to provide two tubes to balance the centrifuge rotor.

*Use a syringe and needle to retrieve the solutions through the silicone stopper, if not all contents are used.

Depending on the volume ejaculate to process, different sizes of tubes and volumes can be used. See table below.

Ejaculate size	Tube size (mL)	BoviPurer layer (mL)	Extended ejaculate (mL)
Small	10-15	4	1-3
Medium	50	10	3-4
Larger	50	20	7-8

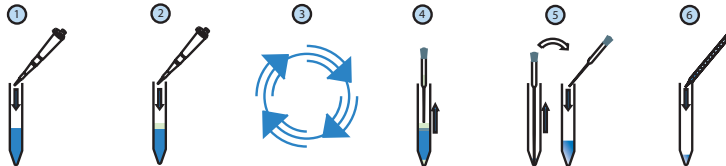
Components (for 10 mL)	Dilution titers
BoviPure™	8 mL
BoviDilute™	2 mL

**To achieve the correct g force:

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

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 300 x g when radius = 165 mm the centrifuge speed must be:

$$Rpm = \sqrt{[300 / (1.118 \times 165)] \times 10^3} = 1275$$



BoviPure Double Layer Fresh Semen

MATERIALS REQUIRED

- BoviPure™
- BoviDilute™
- BoviWash™
- Conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor

HOW TO USE A DOUBLE LAYER BOVIPURE FOR FRESH SEMEN

Important Notes:

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

- BoviPure **does not contain antibiotics**, use aseptic procedures*.

- Prepare the appropriate amount of solutions according to the table below.
- Bring all materials to room temperature.
- Extend the ejaculate with BoviWash™ 1:1 (max conc 250 million/mL).

1. Using a sterile pipette, transfer 80% diluted BoviPure™ to a conical centrifuge tube (see volumes and sizes below).
2. Using a new sterile pipette, layer 40% BoviPure™ carefully over the BoviPure 80%, taking care not to disrupt the gradient layers.
3. Layer extended semen on top of the gradient taking care not to disrupt the layers.
4. Centrifuge at 300 x g for 25-30 minutes** at room temperature, do not use the brake. (longer time for larger volumes).
5. Carefully remove ejaculate, BoviPure Top Layer and most of the BoviPure Bottom Layer.
6. Using a new sterile pipette, transfer and resuspend sperm pellet in 1 mL BoviWash in a new sterile tube (10-15 mL).
7. Dilute to desired sperm concentration with BoviWash™.

Note: we recommend preparing two BoviPure™ preparations for each

sample, to reduce the risk of overloading a single gradient and to provide two tubes to balance the centrifuge rotor.

Depending on the volume ejaculate to process, different sizes of tubes and volumes can be used. See table below.

Ejaculate size	Tube size (mL)	Bottom layer (mL)	Top layer (mL)	Extended ejaculate (mL)
Small	10-15	2	2	1-3
Medium	50	5	5	3-4
Larger	50	10	10	7-8

*Use a syringe and needle to retrieve the solutions through the silicone stopper, if not all contents are used.

	Dilution titers	
Components (for 10 mL)	Top Layer (40%)	Bottom Layer (80%)
BoviPure™	4 mL	8 mL
BoviDilute™	6 mL	2 mL

**To achieve the correct g force:

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

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 300 x g when radius = 165 mm the centrifuge speed must be:

$$\text{Rpm} = \sqrt{[300/(1.118 \times 165)]} \times 10^3 = 1275$$



BoviPure Double Layer Frozen Semen

MATERIALS REQUIRED

- BoviPure™
- BoviDilute™
- BoviWash™
- Micro centrifuge tubes 1.5 mL
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Micro Centrifuge

HOW TO USE A DOUBLE LAYER BOVIPURE FOR FROZEN SEMEN

Important Note: BoviPure **does not contain antibiotics**, use aseptic procedures*.

- Prepare the solutions according to the table below.
- Close the tubes and incubate for at least 30 minutes in 38°C.

1. Using a sterile pipette tip, transfer 500 µL BoviPure 80% to the micro centrifuge tube.
2. Using a new sterile pipette tip, transfer 500 µL BoviPure 40% on top of the bottom layer. Be careful no to disrupt the layers.
3. After thawing the straw (0.25ml), clean the straw and empty its content slowly onto the BoviPure gradient. Assess initial sperm motility from a small sample of the thawed semen.
4. Centrifuge at 300 x g for 15 minutes** at room temperature, do not use the brake.
5. After centrifugation, remove the supernatant carefully, making sure only the pellet remains.
6. Transfer the pellet to a new tube containing 1 ml of BoviWash solution (resuspend carefully).
7. Centrifuge the tube at 300 x g for 5 minutes.
8. Remove the supernatant carefully, leaving the pellet.

Recommendations for IVF

1. Measure the volume of the pellet. Remove 5 µl of the pellet and add to 250 µl of IVF medium in order to assess final motility. Add another 5 µl to 250 µl of water in order to assess sperm concentration in a counting chamber (eg. Neubauer).

2. Calculate the correct dilution to obtain the desired concentration in the IVF drop (1x10⁵ spztz/ml).

3. Incubate the IVF drops and add 10 µl of the diluted pellet.

4. Wash the oocytes twice in IVF medium and transfer to the drops containing the spermatozoa.

5. Finally, place the dish in the incubator for 18-22 hours and continue with embryo culture.

*Use a syringe and needle to retrieve the solutions through the silicone stopper, if not all contents are used

	Dilution titers	
Components	40%	80%
BoviPure™	200 µL	400 µL
BoviDilute™	300 µL	100 µL

**To achieve the correct g force:

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

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 300 x g when radius = 80 mm the centrifuge speed must be:

$$Rpm = \sqrt{[(300/(1.118 \times 80))] \times 10^3} = 1800$$

