

**BoviPure™ System**  
**-For the purification of bovine sperm-**



## BoviPure™ Overview

BoviPure™ is a commercially available reagent designed for use in density gradient centrifugation protocols. It should be diluted with BoviDilute™ to achieve the desired concentration, enabling the creation of one-layer or two-layer gradients. These gradients facilitate the separation of spermatozoa based on density, ensuring that only morphologically normal, motile, and viable spermatozoa are recovered in the pellet after centrifugation.

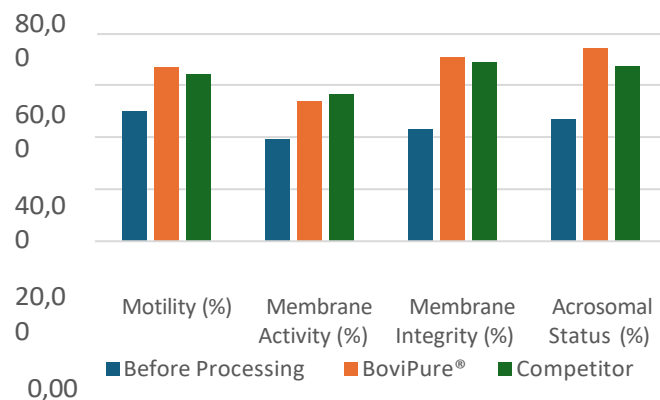
## Storage and stability

BoviPure™ undergoes autoclaved sterilization to ensure aseptic conditions, preventing microbial contamination. It is stable for up to 24 months when unopened and stored between 2–40°C, providing reliable and consistent performance. To maintain optimal performance, it is critical to avoid exposure to temperatures outside this range. Under these storage conditions, BoviPure™ have a shelf life of two years, with the expiry date indicated on the product label. Once opened, bottles should be resealed and stored under aseptic conditions at a temperature range of 2°C to 8°C. Expiring dates applies after opening if stored correctly. Notably, BoviPure™ does not contain any antibiotics, unstable additives, or preservatives, it is important to work aseptically.

## Scientific findings

Recent research has shown that BoviPure™ significantly enhances several key sperm quality parameters: Motility: The percentage of motile spermatozoa increased from 50% to 66.67% after processing with BoviPure™. Membrane Activity: Membrane activity improved from 39.05% to 53.78%, indicating a positive impact on sperm vitality.

Membrane Integrity: The integrity of the sperm membrane increased significantly, rising from 42.97% to 70.85% after gradient separation, demonstrating improved membrane stability. Acrosomal Status: The proportion of live spermatozoa with intact acrosomes increased from 46.90% to 74.16%, a crucial factor for successful fertilization. In conclusion, BoviPure™ provides substantial improvements across several sperm quality parameters, enhancing its potential to improve reproductive



outcomes. BoviPure™ can be used prior to semen sexing procedures. Removing all cells other than the viable spermatozoa, makes the sexing procedures more efficient and faster.

## BoviPure™ Composition

Silane coated silica, Calcium chloride, Potassium chloride, Sodium chloride, Purified water, HEPES, EDTA, Glucose



## **BoviDilute™ Overview**

Is an isotonic salt solution designed for bovine sperm purification protocols. It functions as a diluent for BoviPure™, a colloidal silica-based density gradient medium, enabling the creation of one- or two-layer gradients during centrifugation. This system effectively separates and purifies normal spermatozoa, with the resulting pellet containing purified sperm post-centrifugation.

## **Storage and Stability**

Store unopened bottles at between 2 – 40°C. Avoid temperatures above or below these values. When stored at these temperatures, BoviDilute have a shelf life of 2 years. Open and close bottles under aseptic conditions. After opening, store the bottles at between 2 – 8°C the shelf life remains of 2 years. Expiring dates applies after opening it stored correctly. No antibiotics, unstable additives, or preservatives have been added by the manufacturer to BoviDilute, it is important to work aseptically.

## **Scientific findings**

BoviDilute™ enhances sperm survival by maintaining osmotic equilibrium and supporting optimal gradient formation. This minimizes osmotic stress, which can otherwise harm spermatozoa, and ensures that only viable sperm are isolated during the centrifugation process.

## **Physiological Compatibility**

BoviDilute™ maintains osmotic balance and a stable pH to prevent sperm dehydration or swelling, preserving their viability. It is also additive-free, containing no antibiotics or preservatives, ensuring optimal sperm quality during the process.

## **Gradient Optimization**

When combined with BoviPure™, BoviDilute™ forms one- or two-layer gradients that efficiently separate viable spermatozoa from impurities, with reduced viscosity enhancing gradient formation and improving sperm recovery.

## **Centrifugation Efficiency**

During centrifugation, the pellet formed contains purified spermatozoa with reduced DNA fragmentation. BoviDilute™ also minimizes oxidative stress by isolating spermatozoa from seminal plasma, thereby reducing their exposure to reactive oxygen species, which can negatively impact sperm quality.

## **Sterility and Stability**

BoviDilute™ undergoes autoclaved sterilization to ensure aseptic conditions, preventing microbial contamination. It is stable for up to 24 months when unopened and stored between 2–40°C, providing reliable and consistent performance.

Studies show that gradients prepared with BoviDilute™ result in spermatozoa with improved motility, better viability, and reduced DNA fragmentation, which are all critical factors in optimizing sperm quality for bovine ART. BoviDilute™ enhances the extension process by providing both physiological stability and sterile processing conditions, which are essential for maintaining high-quality semen. While BoviDilute™ does not contain antibiotics, its sterilization process, along with its isotonic composition, effectively minimizes contamination risks. This makes it particularly valuable in density gradient protocols, where high-quality sperm purification is essential for ART procedures. In conclusion, BoviDilute™ is critical in optimizing sperm survival, ensuring both the immediate quality of spermatozoa during centrifugation and the long-term viability of semen during storage, thus contributing significantly to successful bovine ART outcomes.

## BoviDilute™ Composition

Sodium Chloride, Sodium Citrate, Potassium Chloride, HEPES, Calcium Lactate, EDTA, Sodium Pyruvate, Glucose, Purified



## BoviWash™ Overview

Is a medium for extending sperm prior to gradient treatment, and for washing the sperm pellet after density gradient separation. BoviWash™ minimizes sudden pH and osmotic changes during the transition from semen sample to fertilisation medium, maximizes the yield of motile sperm, and improves fertilisation potential.

## BoviWash™ serves two main functions:

1. **Sperm Extension:** It's used to dilute sperm samples before density gradient separation, ensuring that sperm concentration is optimal for the next steps in processing.
2. **Pellet Washing:** After centrifugation during density gradient separation, BoviWash™ is used to resuspend the sperm pellet. This helps remove any residual gradient medium and non-motile sperm, ensuring that only viable, motile sperm are retained. One of the key benefits of BoviWash™ is that it enhances motile sperm yield and improves the fertilization potential by maintaining sperm viability throughout handling. BoviWash™ also optimizes sperm protection and functionality during processing protocols like density gradient separation and washing.

## Product Specifications:

- **Sterility:** It is aseptically filtered to ensure contamination-free use.
- **Compatibility:** BoviWash™ is designed to work seamlessly with the BoviPure™ System, where it is used post-centrifugation to resuspend sperm pellets.

**Scientific Insights:** BoviWash™ contributes to fertilization potential through its osmotic and pH stability. Its isotonic composition, which includes components like sodium chloride and potassium chloride, helps minimize osmotic stress during sperm resuspension after centrifugation. This preservation of osmotic balance is crucial for maintaining membrane integrity and motility, both of which are vital for fertilization capacity. Additionally, BoviWash™ likely contains antioxidant and protective components, such as bovine serum albumin (BSA) and HEPES buffer. These ingredients help mitigate oxidative stress, a major factor in sperm DNA damage and reduced motility. By protecting cellular function, BoviWash™ enhances sperm capacitation and fertilization rates, much like other media that contain antioxidants, such as Urolithin A. When it comes to efficient sperm pellet washing, post-density gradient separation, BoviWash™ plays a significant role in removing seminal plasma and debris. This reduction in oxidative stressors improves the efficiency of sperm selection and enhances the overall quality of the sperm, much like protocols where motility enhancers or antioxidants are used for IVF. Furthermore, BoviWash™ supports IVF workflows by maintaining sperm viability during handling. It is crucial for protocols like IVF, which require high-quality sperm to maximize embryo production. Studies have shown that optimized sperm processing directly correlates with increased fertilization and pregnancy rates. Although direct evidence for BoviWash™ may not be explicitly mentioned in the provided data, its design mirrors the principles established in sperm selection and media optimization studies. These studies emphasize stability, protection, and compatibility, all of which are essential for successful assisted reproductive technologies.

## Applications

BoviWash™ is commonly utilized in bovine reproductive biotechnologies, though its principles may extend to other species requiring sperm preparation for in vitro fertilization or cryopreservation. In conclusion, BoviWash™ plays a crucial role in ensuring high-quality sperm preparation by optimizing sperm protection, maintaining viability, and improving fertilization outcomes in ART processes.

## BoviWash™ Components

Sodium Chloride, Potassium Chloride, Magnesium Sulphate, Potassium Dihydrogen Phosphate, Sodium Bicarbonate, purified water, bSA (Bovine Serum Albumin), Glucose, Calcium Lactate, Sodium Pyruvate, EDTA,



## 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 BoviPure™

Sodium Chloride, Potassium Chloride, Glucose, EDTA, HEPES, Calcium Chloride, Silane-coated silica

## Components BoviDilute™

Sodium Chloride, Potassium Chloride, Glucose, Pyruvate, EDTA, HEPES, Sodium Citrate, Calcium Lactate

## Components BoviWash™

Sodium Chloride, Potassium Chloride, Glucose, Pyruvate, EDTA, HEPES, Magnesium Sulphate, Potassium dihydrate, phosphate, Sodium Bicarbonate, Calcium Lactate, BSA

### Performance Characteristics

#### BoviPure™ System

pH	7.4 - 8.5
Osmolality (mOsm/kg H <sub>2</sub> O)	290 – 320
Endotoxin transfer during treatment	<1.0 EU/mL
Sperm survival 18 hours after	>70%

#### Performance Characteristics BoviWash™

pH	7.4-8.5
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Bottles and stoppers are M.E.A tested.	

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## Storage and Stability

- Bottles and stoppers are M.E.A. tested. 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](http://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.

## 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

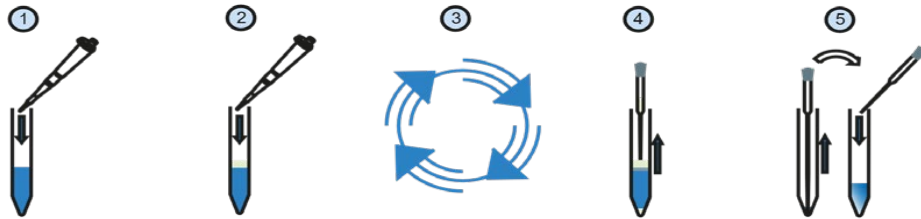
## HOW TO USE A SINGLE LAYER BOVIPURE FOR FRESH SEMEN

### 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\*.
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- 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™.
- 7) 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)	BoviPure™	Extended ejaculate (mL)
Small	10-15	4	1-3
Medium	50	10	3-4
Large	50	20	7-8
			Dilution titers 80%
Components (for 10 mL)			
BoviPure™			8 mL
BoviDilute™			2 mL

\*\*To achieve the correct g force:  $Rpm = \sqrt{\left[ \frac{g}{(1.118 \times r)} \right]} \times 10^3$   
 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:  $Rpm = \sqrt{\left[ \frac{300}{(1.118 \times 165)} \right]} \times 10^3 = 1275$

# BoviPure Double Layer Fresh Semen

## MATERIALS REQUIRED

- Bovi Pure™
- Bovi Dilute™
- 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 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 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).

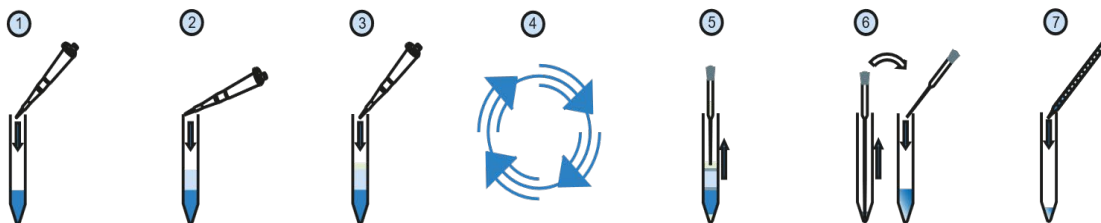
5) Carefully remove ejaculate, BoviPure Top Layer and most of the BoviPure Bottom Layer.

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

Large	50	10	10	7 – 8
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	Dilution Titters	
Components (for 10 mL)	Top Layer (40 %)	Botom Layer (80%)
BoviPure™	4 mL	8 mL
BoviDilute™	6 mL	4 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:  $Rpm = \sqrt{[(300/(1.118 \times 165))] \times 10^3} = 127$

## 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\*.

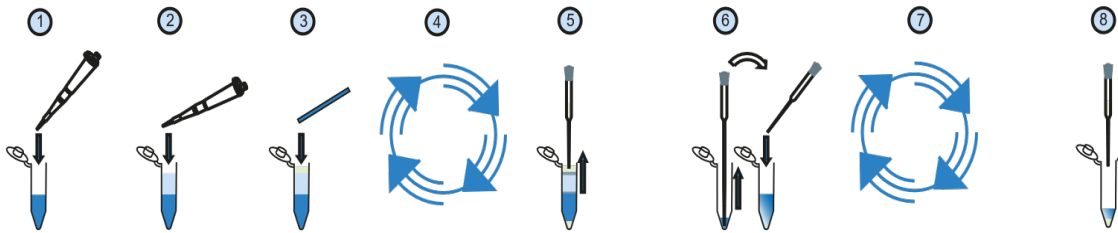
- Prepare the solutions according to the table below.
- Close the tubes and incubate for at least 30 minutes in 38°C.

- 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
- 4) initial sperm motility from a small sample of the thawed semen.
- 5) Centrifuge at 300 x g for 15 minutes\*\* at room temperature, do not use the brake.
- 6) After centrifugation, remove the supernatant carefully, making sure only the pellet remains.
- 7) Transfer the pellet to a new tube containing 1 ml of BoviWash solution (resuspend carefully).
- 8) Centrifuge the tube at 300 x g for 5 minutes.
- 9) 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 ( $1 \times 10^6$  spz/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.
- 6) \*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™	4 µL	8 µL
BoviDilute™	6 µL	4 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 = 165 mm the centrifuge speed must be:  $Rpm = \sqrt{[ (300 / (1.118 \times 165)) ] \times 10^3} = 1275$

## References.

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*S.L. Speckhart et al., STAR protocols, 2023-03, Vol.4 (1), p.101924-101924,*

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*Arias ME et al, Bovine sperm separation by Swim-up and density gradients (Percoll and BoviPure):*

*Effect on sperm quality, function and gene expression*

*Arias ME et al, Reproductive Biology 17 (2017) pp 126–132*



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### Order Information

BP-100 BoviPure™ 100 mL

BD-100 BoviDilute™ 100 mL

BW-100 BoviWash™ 100 mL



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