

# PureSperm® Buffer

## Intended Use: Summary and Explanation

PureSperm®Buffer is a sterile (autoclaved SAL  $10^{-3}$ ) isotonic salt solution. It is optimised for the dilution of PureSperm®100 in the preparation of density gradients for the separation and purification of human sperm for use in ART. This gradient system effectively separates normal sperm from lymphocytes, epithelial cells, abnormal or immature sperm, cell debris, bacteria and seminal fluid.

## Components

Sodium chloride	EDTA
Potassium chloride	Glucose
Sodium citrate	Purified water
Calcium lactate	
Sodium pyruvate	
HEPES	

## Performance Characteristics

pH:	7.4-7.8
Osmolality (mOsm/kg H <sub>2</sub> O)	300-310
Endotoxin levels	<1.0 EU/mL
Sperm survival 18 hours after density gradient separation	>70%

Contents are tested by human sperm survival only

Bottles and stoppers are 2-cell M.E.A. tested

## Storage and Stability

Store unopened bottles at 2 to 40°C and avoid temperatures above or below these values. Under these conditions PureSperm®Buffer 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.

No antibiotics, unstable additives or preservatives have been added by the manufacturer to PureSperm®Buffer.

## Precautions and Warnings

- Use aseptic procedures at all times
- If available, use sealed buckets during centrifugation to avoid creation of aerosols
- PureSperm®Buffer does not represent any 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 use contents if tamper-evident seal is broken
- Federal Law (USA) restricts this device to sale by or on the order of a physician
- Please check for regulatory compliance governing the use of ART products in your country

## Ordering Information

<b>Volume</b>	<b>Article No.</b>
100 mL	PSB-100

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



[www.nidacon.com](http://www.nidacon.com)



  
**Nidacon**  
International AB

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](mailto:contact@nidacon.com)  
[www.nidacon.com](http://www.nidacon.com)

## Recommendations

Prepare two PureSperm® gradients for each semen sample. This reduces the risk of overloading a single gradient, provides security when handling tubes or recovering sperm pellets and provides two tubes to balance the centrifuge rotor.

## Reagents and Equipment

- PureSperm®100, PureSperm®Buffer and PureSperm® Wash
- Bench top centrifuge with swing out rotor
- Disposable sterile conical centrifuge tubes
- Sterile pipettes

## Procedure for preparation of PureSperm® gradients and sperm separation

Bring all solutions to room temperature.

1. Mix 2mL PureSperm®Buffer with 8mL PureSperm® 100 to form 10mL 80% PureSperm®
2. Mix 6mL PureSperm®Buffer with 4mL PureSperm® 100 to form 10mL 40% PureSperm®
3. Use a pipette with a sterile tip to add 2 mL of 80% PureSperm® to a conical centrifuge tube
4. Use a new sterile pipette tip to carefully layer 2mL 40% PureSperm® on top of the 80% PureSperm®
5. Use a sterile pipette to carefully layer liquefied semen (up to 1.5mL) onto the PureSperm® gradient
6. Centrifuge at 300 x g for 20 minutes. Do not use the brake. Calculate the correct RPM for your centrifuge
7. Use a new sterile Pasteur pipette and aspirate, in a circular movement from the surface, everything except the pellet and 4-6mm of 80% PureSperm®. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5mL
8. Use a new sterile Pasteur pipette to aspirate the pellet (or the lowest 0.5mL liquid). Transfer sperm pellet to new tube and resuspend pellet in 5mL PureSperm® Wash.
9. Centrifuge at 500 x g for 10 minutes. Do not use the brake
10. Aspirate PureSperm®Wash supernatant leaving as little liquid as possible above pellet. If no pellet is seen, leave the bottom 0.25mL fluid
11. Resuspend the sperm pellet in a suitable volume of culture medium to obtain the required sperm concentration. The sperm sample is now ready for analysis or use

