Intended Use
Stable colloid/isotonic salt solution for preparation of density gradient used to separate human sperm.

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

Performance Characteristics
- pH: 7.4-7.8
- Osmolality (mOsm/kg H₂O): 300-310
- Endotoxin transfer during treatment: <1.0 EU/mL
- Human sperm survival 18 hours after density gradient separation: >70%

Precautions and Warnings
- When retrieving the sperm pellet, follow the instructions given in the 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. PureSperm®100 causes floors and benches to be extremely slippery
- PureSperm®100 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 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.
- 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

Storage and Stability
Store unopened bottles at 2 to 27ºC and avoid temperatures above or below these values. Under these conditions PureSperm®100 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 8ºC 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®100.

Ordering Information
- Volume
  - 100mL
  - 250mL
  - 4x250mL
- Article No.
  - PS100-100
  - PS100-250
  - PS100-1000K

For further technical information or assistance, please contact your distributor or the manufacturer.
**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. Add 2mL PureSperm® Buffer to 8mL PureSperm®100 to form 10mL 80% PureSperm®
2. Add 6mL PureSperm® Buffer to 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®
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 0.25mL
8. Use a new sterile Pasteur pipette to aspirate the pellet (or the lowest 0.25mL 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. Calculate the correct RPM for your centrifuge
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

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