# PureSperm® Buffer

**Intended Use**

Balanced salt solution optimised for the dilution of PureSperm®100 to prepare layers for density gradients.

**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 H2O) | 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 M.E.A. tested

**Storage and Stability**

Store unopened bottles at 2 to 40ºC 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 8ºC 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 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)
- 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
- Do not re-use. Reuse may result in biological contamination and/or property changes in the product
- 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**

| **Volume** | **Article No.** |
|---|---|
| 100 mL | PSB-100 |

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

Temperature limit

Use by - see label

STERILE — Sterilized using aseptic processing techniques

LOT — Batch code

Consult instructions for use

CE Mark (Conformité Européen)

Manufacturer

**www.nidacon.com**

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Nidacon

INS-PSB100-YA/16

**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. 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

**Procedure for preparation of a viscous sample**

Bring all solutions to room temperature.

1. Measure the volume of the semen sample
2. Dilute 1+3 , 1 part PureSperm®Buffer and 3 parts sample (e.g. 0.5 ml PureSperm®Buffer + 1.5 ml semen sample)
3. Incubate at 37°C for 15-30 minutes
4. Mix using a pipette
5. Ready for sperm preparation on a density gradient

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