Intended Use

Optimised for freezing human sperm.

Components

- Sodium chloride
- Potassium chloride
- Magnesium sulphate
- Potassium dihydrogen phosphate
- Sodium bicarbonate
- Sodium pyruvate
- Calcium lactate
- EDTA
- HEPES
- Glucose
- Glycerol
- Glucose
- Glycerol
- Water
- Sodium chloride
- Potassium chloride
- Magnesium sulphate
- Potassium dihydrogen phosphate
- Sodium bicarbonate
- Sodium pyruvate
- Calcium lactate
- EDTA
- HEPES
- Glucose
- Glycerol
- Water

Performance Characteristics

- pH: 7.5-8.5
- Endotoxin levels: <1.0 EU/mL
- Recovery rate of the original motile spermatozoa after freezing and thawing: >50%

Contents are tested by human sperm survival only. Bottles and stoppers are M.E.A. tested.

Storage and Stability

Store at 2 to 30°C and avoid temperatures above or below these values. Under these conditions Sperm CryoProtec™ has a shelf-life of 12 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 and handled according to manufacturer’s recommendations.

No antibiotics, unstable additives or preservatives have been added by the manufacturer to Sperm CryoProtec™.

Precautions and Warnings

- Use aseptic procedures at all times
- Sperm CryoProtec™ contains glycerol which is combustible. 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
- 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

Recommendations

Although it is possible to freeze unprocessed semen, we recommend that the ejaculate be prepared on a PureSperm® density gradient and washed with PureSperm®Wash before adding Sperm CryoProtec™. This method removes seminal plasma as well as ROS and their sources, thereby ensuring optimal recovery of motile sperm after thawing.

Reagents and Equipment

- Sperm CryoProtec™ and PureSperm®Wash
- CryoFloater™
- Sterile pipettes
- Disposable sterile conical centrifuge tubes (e.g. Falcon 2075)
- Disposable sterile cryopreservation vials or plastic straws

Ordering Information

<table>
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<tr>
<th>Volume</th>
<th>Article No.</th>
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<td>2x20 mL</td>
<td>SCP-020</td>
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For further technical information or assistance, please contact your distributor or the manufacturer.

Manufacturer:
Nidacon, Flöjelbergs gatan 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
Freezing sperm with Sperm CryoProtec™

1a) Gradient-prepared sperm
1-2. Add 1 part of Sperm CryoProtec™ to 3 parts of sample (e.g. 100μL SCP to 300μL sperm sample), ensuring thorough mixing after adding each drop in order to avoid osmotic shock.
3. Fill straws with sperm suspension and seal the straws.
4. Equilibrate straws in refrigerator for 10-60 minutes.
5. Place the straws horizontally in nitrogen vapour on the CryoFloater™Straws. Close lid. Leave for 10-30 minutes.
6. Transfer the straws quickly into the liquid nitrogen and store in liquid nitrogen. Do not touch the straws with your hand.

1b) Thawing of gradient-prepared sperm
1. Remove straw from liquid nitrogen tank.
2. Place straw in water at 37°C for 30 secs.
3. Dry surface of straw.
4. Cut one end of straw.
5. Resuspend contents in 5mL PureSperm®Wash by cutting the upper end of the straw. Any sperm suspension remaining in the straw can be expelled using a pipette.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Aspirate PureSperm®Wash supernatant leaving as much liquid as required for desired concentration. If no pellet is seen, leave.

2a) Unprocessed ejaculate
1-2. Add 1 part of Sperm CryoProtec™ to 3 parts of sample (e.g. 100μL SCP to 300μL sperm sample), ensuring thorough mixing after adding each drop in order to avoid osmotic shock.
3. Transfer 0.8-1.8 ml of the mixture to 2 ml cryovials.
4. Place the vials in the fridge (4-5°C) for 30 min.
5. Freeze the vials horizontally in the freezer or in nitrogen vapour, above the liquid nitrogen surface on the CryoFloater™Vials. Close lid. Leave for 30 min.

2b) Thawing of unprocessed ejaculate
1. Remove the vials from the liquid nitrogen tank.
2. Place vials in water at 37°C until no ice crystals can be seen, approximately 2-3 min.
3. Dilute the thawed material with 0.5 ml PureSperm®Wash.
4. Prepare a 40% and 80% PureSperm® density gradient, with 1 mL in each layer. Layer the thawed ejaculate onto the gradient.
5. Centrifuge at 300 x g for 20 min.
6. Aspirate everything except the pellet and 4-6 mm of the PureSperm® 80% layer.
7. Use a new pipette to aspirate the pellet. Transfer to a new tube containing 4 ml PureSperm®Wash.
8. Centrifuge at 500 x g for 10 min.
9. Aspirate PureSperm®Wash supernatant and the sample is now ready for use.