ENHANCE Sperm Freeze

Reference#: 15609 (20mL), 15765 (100mL)

For laboratory use only; other uses must be qualified by the end user

INTENDED USE

Enhance Sperm Freeze is a HEPES buffered freezing medium for use with human sperm[1, 2]. Enhance Sperm Freeze contains 0.4 % human serum albumin (HIV and Hepatitis negative) to protect the sperm from damage due to the freezing procedure[3]. The bottle of Enhance Sperm Freeze contain reagent for freezing 6 to 10 samples.

ENHANCE SPERM FREEZE MEDIUM AND SPERM PREPARATION

Before freezing

In case of very low sperm concentrations it is advisable to concentrate the sperm before freezing. This may increase sperm quality after thawing and will reduce the number of straws / vials to be frozen.

After thawing

Artificial insemination: Enhance Sperm Freeze / semen mixture can be used directly for insemination. No further preparations are necessary. All other cases: use sperm preparation techniques after thawing the semen to eliminate dead sperm cells and debris. Dilute the concentrated sperm in washing medium or any other medium you would normally use.

PRODUCT SPECIFICATIONS AND QUALITY CONTROL

pH: 7.20 - 7.60
Sterility: sterile
Endotoxin: < 0.25EU/mL
Sperm survival test: ≥ 80% survival after 4-hour exposure of untreated semen to the test medium
Not MEA tested
A Certificate of Analysis is provided with every lot of Enhance Sperm Freeze Medium.

WARNINGS BEFORE USE

- Do not use the product if:
    - It becomes cloudy, or shows any evidence of microbial contamination;
    - Seal of the container is opened or defect when the product is delivered;
    - Expiry date has been exceeded;
- Do not freeze before use.
- Do not re-sterilize after opening.
- Depending on the number of procedures that will be performed on one day, remove the required volume of medium under aseptic conditions in an appropriate sterile recipient. This is to avoid multiple openings/warming cycles of the medium. Discard excess (unused) media.
- Keep in its original packaging until the day of use

RECOMMENDED METHOD

Ensure all media are well mixed and at room temperature before use.

Freezing

1. Allow the semen to liquefy at room temperature for 30 minutes.
2. Use a syringe and needle to remove the desired quantity of Enhance Sperm Freeze from the vial; this will reduce the risk of contamination of the medium. *NOTE: it is not necessary to completely remove the metal cap when using a syringe and needle.*
3. Mix 1 ml of sperm with 0.7 ml of Enhance Sperm Freeze. Add the medium in drops while gently swirling. Caution: to avoid cold-shock, make sure Enhance Sperm Freeze is at room temperature.
4. Leave the mixture for 10 minutes at room temperature for equilibration.
5. Label the cryopreservation device with patient information.
6. Load the sample/medium mixture into the freezing device.
7. For cryopreservation straws, leaving approximately 1.5 cm of air at the end of the straw. Seal straws according to manufacturer's recommendations. Shake to move the air-bubble to the center of the straw.
    7.1. Freeze straws horizontally on a 1-3 cm styrofoam board for 15 minutes, in a liquid nitrogen bath to allow for freezing in vapor phase.*

8. For cyrovials load the semen mixture; Do not fill cryovials completely to allow for expansion.
   8.1. Freeze cryovials vertically on a 1-3 cm styrofoam board for 30 minutes, in a liquid nitrogen bath to allow for freezing in vapor phase.*
9. Once frozen, the straws / vials are immersed and stored in liquid nitrogen in secure tanks at a temperature of -196° C (-371° F).

* Optional, this step can be performed using a slow-freeze machine programmed for sperm freezing.

Thawing

1. Remove as many straws or cryovials as required from the liquid nitrogen.
2. Place straws in a water bath at 35 ± 2 °C for 30 seconds.
   2.1. Cut off the end of the straw, place the open end inside a clean container (e.g. a test tube) and tap the straw against the side of the container to allow complete evacuation of the mixture.
3. Place cryovials in a water batch at 35 ± 2 °C for 10 minutes.
   3.1. Transfer the semen mixture from cryovial to a clean container ((e.g. a test tube).
4. Dilute the concentrated sperm in a suitable medium (at least 3mL media per 0.5ml semen) and mix thoroughly
5. If required, continue with gradient separation according to specifications.

MATERIAL NOT INCLUDED

Cryopreservation straws, test tubes or cryovials, liquid nitrogen, syringe and needle.

STORAGE/DISPOSAL INSTRUCTIONS

- Store between 2-8 °C.
- Keep away from (sun)light.
- The product can be used up to 7 days after opening, when sterile conditions are maintained, and the products are stored at 2-8 °C.
- Stable after transport (max. 5 days) at elevated temperature (≤ 37 °C).
- The devices need to be disposed in accordance with local regulations for disposal of medical devices.

WARNING AND PERCAUTIONS

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens.

CAUTION: All human derived fluids should be treated as potentially infectious. Source materials from which this product was derived was found negative when tested for antibodies to HIV, HBc, HCV, and HTLV I/II and non-reactive for HBsAg, HCV RNA, and HIV-1 RNA and syphilis. No known test method can offer assurance that products derived from human blood will not transmit infectious agents.

REFERENCES

1. Mahadevan M, Trounson AD. (1983) Effect of cryoprotective media and dilution methods on the preservation of human spermatozoa. Andrologia, 15: 355-66.
2. Mahadevan M, Trounson AD, Leeton JF. (1983) Successful use of human semen cryobanking for in vitro fertilization, Fertil Steril, 15: 355-66.
3. Brotherton J. (1990) Cryopreservation of human semen. Archives of Andrology, 25: 181-95
4. Kobayashi T, Kaneko S, Hara I, Park YJ, et al. (1991) Concentrating human sperm before cryopreservation. Andrologia, 23: 25-8.
5. Graczykowski JW, Siegel MS. (1991) Influence of sperm processing on the fertilizing capacity and recovery of motile sperm from thawed human semen. Archives of Andrology, 26: 155-61.