

SPERM FREEZING

Sperm freezing when using straws

Directions for supplementation of un-supplemented G-Series™ media can be found in the G-Series Manual on www.vitrolife.com.
Once supplemented, the media should be used as the G-Series PLUS media described below.

Freezing



1. Assess the semen sample

Ensure that both liquified semen and SpermFreeze Solution are at

ambient temp

Measure the total volume of semen and carry out semen analysis as required.



2. Add SpermFreeze Solution

Dilute with equal volume of semen and SpermFreeze Solution. Add SpermFreeze Solution slowly and dropwise to the semen and then carefully tilt after each drop added. Close the lid tightly and turn the tube upside down 20 times, being careful not to create bubbles.



3. Leave in room temperature

> 10 min

4. Load straws

Mark straws with patient ID and load the semen mixture. Ensure that some air space is left in the lower part of the straw. Seal the straw according to the straw manufacturer's instructions.



5. Freezing

Place the straws horizontally on a 1-3 cm styrofoam board in a liquid nitrogen bath. Leave for

30 min



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

6. Store in N₂(l)

Transfer the straws quickly into liquid nitrogen and store at

-196 °C

Thawing

The day before thawing:



Pre-equilibrate G-IVF PLUS at

**37°C 6 % CO₂
overnight**

1. Remove straws from N₂(l)

Remove straws from -196 °C and place them in a water bath at

35 ± 2 °C 30 sec

Wipe the straws dry with a clean paper towel and open according to instructions from the manufacturer of the straws.



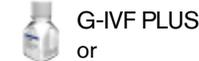
2. Dilute with G-IVF PLUS

Expel semen mixture into clean test tubes and dilute with equal amount of equilibrated G-IVF PLUS. G-IVF PLUS should be added dropwise to the semen mixture and the solution carefully mixed after each addition.



3. Gradient separation

Continue with gradient separation according to the G-series manual.



* Freezing program for machine:
Start temperatur: +20°C
1. -5°C/min to -8°C
2. Hold 1 min
3. -10°C/min to -25°C
4. -25°C/min to -150°C