

# SPERM FREEZING

## Sperm freezing when using cryovials

Directions for supplementation of un-supplemented G-Series™ media can be found in the G-Series Manual on [www.vitrolife.com](http://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 vials

Mark cryovials with patient ID and load the semen mixture into cryovials.



Do not fill cryovials completely to allow for expansion.

#### 5. Freezing

Place the cryovials upright 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 N<sub>2</sub>(l)

Transfer the cryovials quickly into the liquid nitrogen and store at

**-196 °C**

### Thawing

The day before thawing:



Pre-equilibrate G-IVF PLUS at

**37°C 6 % CO<sub>2</sub>  
overnight**

#### 1. Remove cryovials from N<sub>2</sub>(l)

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

**35 ± 2 °C 10 min**

Wipe the cryovials dry with a clean paper towel.



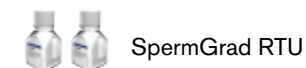
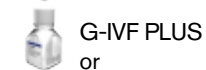
#### 2. Dilute with G-IVF PLUS

Transfer semen mixture to 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