



# Sperm freezing



## Preparation

Ensure all media are well mixed before use.

## Before freezing

In case of very low sperm concentrations it is advisable to concentrate the sperm before freezing. GM501 Gradient can be applied before freezing to remove debris and to enrich the concentration of motile cells in a sample. This may increase sperm quality after thawing and will reduce the number of straws to be frozen. GM501 SpermStore needs to be warmed to room temperature before use to avoid cold-shock.

## Freezing

1. Allow the semen to liquefy at room temperature for 30 minutes.
2. Mix 1.00 ml of sperm with 0.70 ml GM501 SpermStore. Add the medium in drops while gently swirling.
3. Leave the mixture for 10 minutes at room temperature for equilibration.
4. Aspirate the sample/medium mixture into the freezing straws, leaving approximately 1.5 cm of air at the end of the straws.
5. Seal the straws.
6. Dry off individually with a lint-free wipe.
7. Shake to move the air-bubble to the centre of the straw.
8. Place the straw horizontally on a styrofoam board in a liquid nitrogen bath to allow for freezing in vapour phase. Leave for (at least) 15 minutes.
9. Transfer straws quickly into liquid nitrogen and store at -196 °C.

## Thawing

1. Remove as many straws as required from the liquid nitrogen.
2. Place the samples in tap water for 5 minutes.
3. Cut off the end of the straw, place the open end inside a container (e.g. a conical centrifugation tube) and tap the straw lightly against the side of the container to allow complete evacuation of the mixture.
4. Dilute the thawed sperm in a suitable insemination medium (at least 3.0 ml per 0.5 ml semen) and mix thoroughly.
5. Centrifuge for 15 minutes at 300-350x g.
6. Resuspend pellet in a suitable insemination medium.

## After thawing

If necessary, use sperm preparation techniques after thawing the semen to eliminate dead sperm cells and debris.

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### Planer Limited

110 Windmill Road, Sunbury-On-Thames  
Middlesex TW16 7HD, United Kingdom  
A Hamilton Thorne Company

**Tel:** +44 (0)1932 755 000  
enquiries@planer.com  
www.planer.com

### Manufactured by

**Gynemed GmbH & Co. KG**  
Lübecker Straße 9 23738 Lensahn  
Germany

**Tel.:** +49 (0) 4363 90 32 90  
info@gynemed.de  
www.gynemed.de