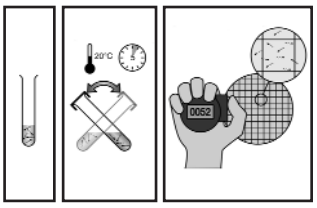
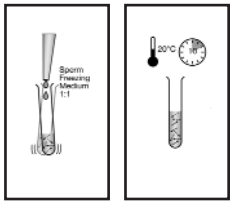
Procedure (Sperm Freezing Medium):

Freezing:

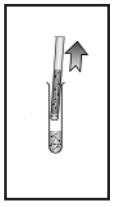


1. After liquefaction, measure the total volume of the ejaculate and carry out semen analysis as required.



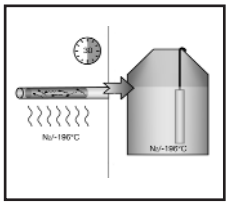
2. Ensure that both semen sample and Sperm Freezing Medium are at room temperature and dilute the semen 1:1 (v/v) with the Sperm Freezing Medium. The medium should be added dropwise to the semen and the solution carefully mixed after each addition.

3. The mixture is left at room temperature for a minimum of ten minutes.



4. Load the diluted semen into straws or cryo-tubes and seal according to the manufacturer's recommendations.

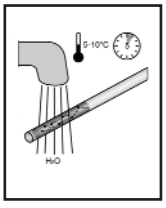
NOTE! It is very important that you leave some air space in the lower part of the straw for sealing as well as to allow expansion of the solution during freezing.



5. Suspend the straws horizontally for 30 minutes, just above the surface of the liquid nitrogen. Cryo-tubes should be attached to a cane and then suspended above the surface of the liquid nitrogen for the same period of time. Alternatively, run the sperm cryopreservation programme available for your freezing machine.

6. Finally, transfer the straws or cryo-tubes into liquid nitrogen and store at -196ºC.

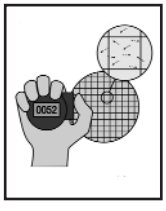
Thawing:



1. Remove straws or cryo-tubes from liquid nitrogen and place them in cold running water for 5 minutes.

2. Open the straws or cryo-tubes according to the manufacturer's instructions and remove the thawed semen.

3. Dilute the semen with Sperm Preparation Medium (1:1) to reduce the toxic effect of glycerol.



4. Quickly evaluate the survival of the sperm. If necessary thaw additional straws for preparation.

5. Immediately prepare sperm by the density gradient method using SupraSperm® (Cat. No 1091/1092 or 1097) or the swim-up procedure using Sperm Preparation Medium (Cat.No. 1069/1070). Please refer to the specific protocol recommended for the use of each product.

Note: Concentrating the sperm prior to freezing can enhance post-thaw recovery of semen samples with low sperm counts. A final concentration of minimum 10 x 10^6/ml is recommended.