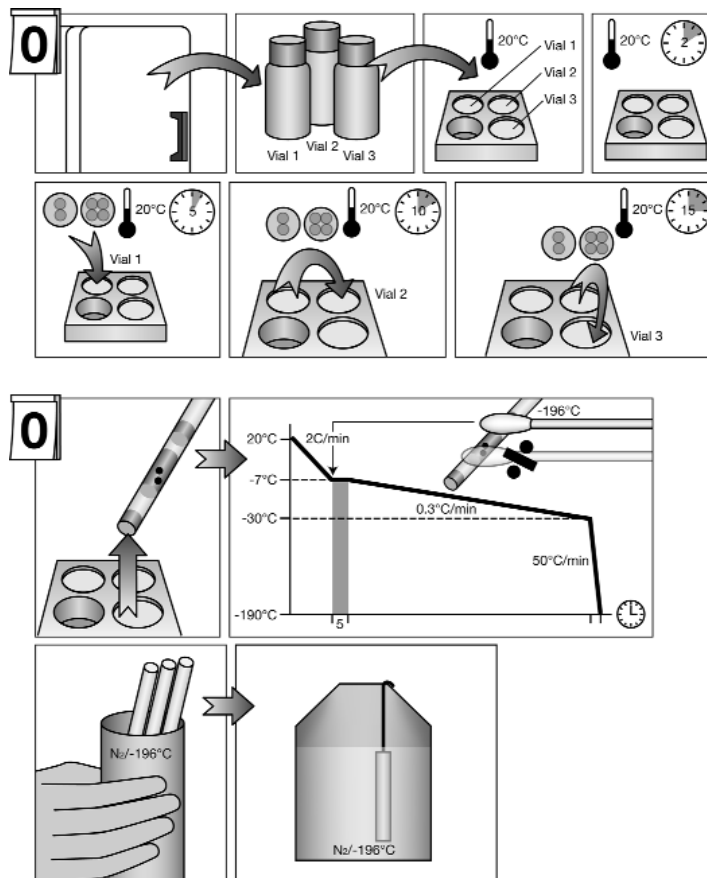
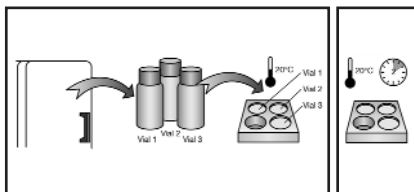


Quick overview

Embryo Freezing pack



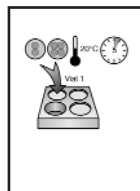
Procedure (Embryo Freezing Pack):



1. Label dishes for freezing solutions and pipette appropriate volumes of freezing solution Vial 1, Vial 2 and Vial 3 into the respective dishes. Equilibrate to room temperature.

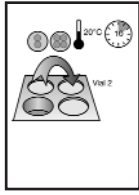
2. Label the cryo straws with unique information. Do not write directly on straws as the solvents can penetrate the straw and harm the pre-zygotes / embryos, but use special tape to protect the straw. If embryo plugs are used, patient information can be directly transferred onto the plugs prior insertion into the straw.

3. Aspirate some of the freezing solution Vial 1 followed by aspiration of the pre-zygotes / embryos from the culture dish.



4. Transfer the pre-zygotes / embryos, with as little culture media as possible, to Vial 1 and start the timer (5 minutes).

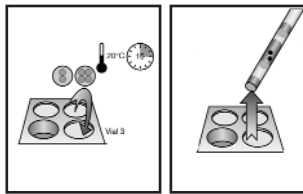
NOTE! The pre-zygotes / embryos will float to the surface of the media and slowly sink to the bottom of the dish. It is therefore an advantage if you carefully "catch" the pre-zygotes / embryos and place them at the bottom of the dish.



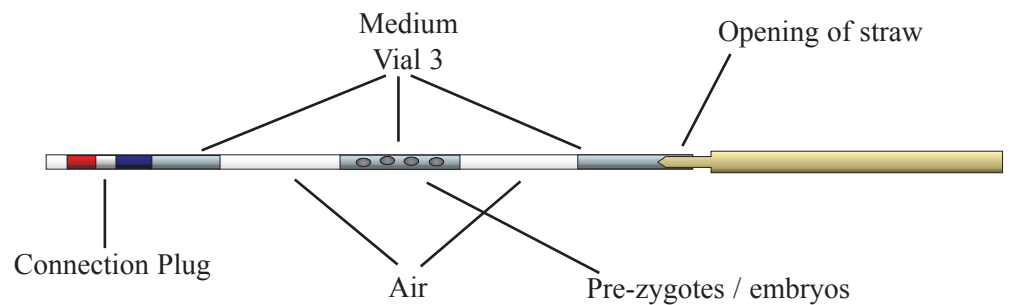
5. Just before the end of the 5 minutes take up some of freezing solution Vial 2 into the pipette. When the timer alarms, carefully take up the pre-zygotes / embryos and transfer to the Vial 2 solution. The pre-zygotes / embryos are left for exactly 10 minutes in the Vial 2 solution.

6. Just before the pre-zygotes / embryos are transferred to the Vial 3 solution the insides of the cryo straws are cleaned with the same solution but from another dish.

NOTE! Take up some air in the syringe, which facilitates removing the surplus Vial 3 solution. Do not allow this solution to come in contact with the plug.

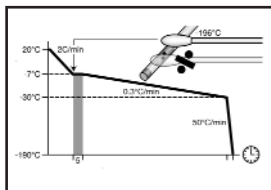


7. Take up Vial 3 solution in the pipette. When the timer alarms, carefully take up the embryos and transfer to the Vial 3 solution. When the pre-zygotes / embryos have sunk to the bottom of the dish, load the right straw with the pre-zygotes / embryos in the same manner as when you perform ET. When the first column of medium touches the plug it will seal, stopping all further movement in the straw (see diagram below).



8. Seal the opening of the straw with an embryo plug, so that liquid nitrogen will not leak inside the straw. Repeat for all pre-zygotes / embryos.

Note! Heat sealing is not recommended as it causes cracking of the straws and the heat releases embryotoxic material from the straws.

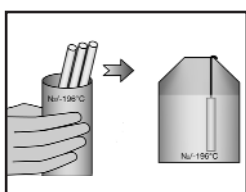


9. Place the straws in the freezing machine and initiate the cooling programme:

- Cool from room temperature to -7°C in steps of 2°C per minute.
- Seed manually and hold the temperature for 5 minutes.

NOTE! When the solution is white seeding has been initiated. Do not seed the straw close to the pre-zygote / embryo and do not drop or shake it. If there are air bubbles in the straw it may reduce pre-zygote / embryo survival.

- Cool from -7°C to -30°C in steps of 0.3°C per minute.
- Cool from -30°C to -190°C in steps of 50°C per minute.
- Transfer the straws directly into liquid nitrogen and store at -196°C .



NOTE! Attention must be paid to the handling of straws at low temperature as they may thaw very quickly.