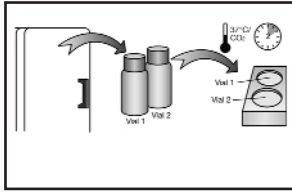
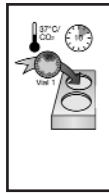


Procedure (BlastFreeze™)

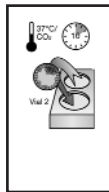


1. Label the dish for freezing solutions and pipette appropriate volumes of freezing solution Vial 1 and Vial 2 into the dish. Equilibrate in CO₂ at 37°C.
2. Label the cryo straws with unique information. Do not write directly on straws as the solvents can penetrate the straw and harm the embryos. Use tape (such as autoclave indicator 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 Vial 1 solution and then the blastocysts from the culture dish.



4. Transfer the blastocysts, with as little culture media as possible, to Vial 1 and place the dish in a CO₂ incubator for 10 minutes.

NOTE! The blastocysts 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 blastocysts and place them at the bottom of the dish.

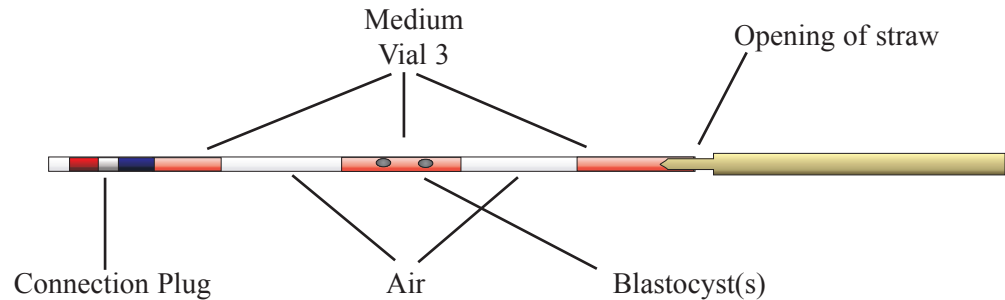


5. Just before the end of the 10 minutes take up some of freezing solution Vial 2 into the pipette. When the timer alarms, carefully take up the blastocysts and transfer to the Vial 2 solution. The dish is again placed in a CO₂ incubator for 10 minutes.
6. Just before the timer alarms, the inner part of the cryo straws are cleaned with freezing solution Vial 2.

NOTE! Take up some air in the syringe, which facilitates removing the surplus Vial 2 solution. Do not allow this solution to come in contact with the plug.

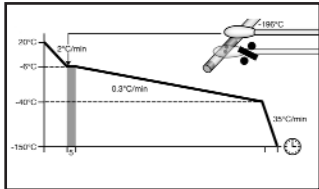


7. Transfer the blastocysts into straws (a maximum of 2 blastocysts per straw) using the Vial 2 medium as transfer medium. The straw is loaded in the same manner as when you perform ET. When the first column of medium touches the plug it will seal, stopping all further movement in the straw (see diagram below).



8. Seal the opening of the straw with a plug, so that liquid nitrogen will not leak into the straw. Repeat for all blastocysts.

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

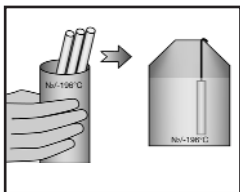


9. Begin the cooling programme as described below:

- Cool from room temperature to -6°C in steps of 2°C per minute.
- Seed manually at -6°C .

NOTE! When the solution is white, seeding has been initiated. Do not seed the straw close to the blastocyst(s) and do not drop or shake it. If there are air bubbles in the straw it may reduce blastocyst survival.

- Cool from -6°C to -40°C in steps of 0.3°C per minute.
- Cool from -40°C to -150°C in steps of 35°C per minute.
- Transfer the straws 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.