



# Slow freezing



## Preparation

Ensure all media are warmed up to room temperature and mixed well before use.

## Freezing

- Using a sterile pipette place 1 ml of Embryo Store Freeze medium in a centre well dish (at room temperature).
- Add the embryos to the freezing medium and allow them to settle for about 30 seconds.  
**Caution: Due to density differences, the embryos tend to float upwards and shrink.**
- Load the embryos in straws leaving about 1/5 air in the straw.
- Seal the straws and label with name, date and number of embryos.
- Start freezing program within 5-10 minutes.  
Below is an example of a freezing protocol.

	Temperature range	Freezing rate	Time
Phase 1	RT to +4 °C	-10 °C/min	2 min
Phase 2	+4 °C to -6 °C	-2 °C/min	5 min
Phase 3	-6 °C (autoseeding)	0 °C/min	10 min
Phase 4	-6 °C to -30 °C	-0.3 °C/min	80 min
Phase 5	-30 °C to -196 °C	-199 °C/min	1 min

## Thawing

- In a 4-well culture dish place 1 ml of each EmbryoStore Thaw thawing solution (1, 2 & 3). This leaves 1 well empty to retrieve the frozen/thawed embryos.
- Prepare a water bath at 37 °C to thaw the straws.  
Prepare a 1 ml tuberculin-syringe by filling it with 0.8 ml of air first followed by 0.2 ml of EmbryoStore Thaw 1 medium.
- Remove the straws from liquid nitrogen and leave at room temperature for about 5 seconds.
- Submerge the straw in the water bath at 37 °C for another 5 seconds (ensure no frozen part remains in the straw).
- Empty the straw by opening both ends of the straw (above the empty well) and blowing the contents of the syringe through the straw.
- Retrieve the embryos and place them in EmbryoStore Thaw 1 thawing solution.
- Transfer the embryos to EmbryoStore Thaw 2 thawing solution after 3-5 minutes.
- After another 3-5 minutes the embryos are transferred to EmbryoStore Thaw 3 thawing solution. Leave for a further 3-5 minutes before proceeding.
- At the final stage the embryos are transferred in IVF culture medium (e.g. GM501 Cult media) for washing and further culture.

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