

Vitrification and Warming Manual of Procedures

Stroebech Vitrification and Warming Media Kits are designed for vitrification and warming of *in vitro* and *in vivo* produced embryos

Our Vitrification Media can be used with all vitrification devices. Due to the large amount of lipid in bovine embryos we recommend the method where the embryos are placed on the tip and plunged directly into the liquid nitrogen, rather than vitrifying on a plate.

Remember:

IVF embryos are NOT in vivo embryos, but more fragile and sensitive to pH and temperature fluctuations

VITRIFICATION

Vitrify embryos in 100 µl drops or in 500 µl volumes in wells a 4-well plate (4WP)

- The vitrification procedure must be performed at 25-30 °C
- Prepare the drops/well with the media: Step 1, Step 2 and Step 3
- Prepare the vitrification device
- Prepare the cryobath or a styro foam box with Liquid Nitrogen
- Vitrification of several embryos can be performed in the same drops/wells if you work fast
- Do not use the same media for different animals
- Inspect the procedure visually, the timings in the table **may vary depending on embryo shrinkage or expansion**

Step 1	Step 2	Step 3	Temperature
2 min	2 min	30 sec	25-30 degrees

- Place the embryo in Step 1 medium set the timer, and move through the 3 steps
- Use as little volume as possible when placing the embryo on the cryo device,
- No more than 0.25 µl
- Plunge immediately into liquid nitrogen
- Seal as indicated on the instructions for use on the device.

WARMING

Warm embryos in 100 µl drops or in 500 µl volumes in wells a 4-well plate (4WP)

The warming procedure must be performed at 35°C.
The media can be warmed in incubator prior to use 38.8°C

- Prepare the drops/well with the media: Step 1, Step 2, Step 3 and Step 4

Step 1	Step 2	Step 3	Step 4	Temperature
3 min	2 min	2 min	1 min	35°C

- Remove cryo device from Liquid Nitrogen tank
- Immediately place the tip with the embryo in Step 1 warming medium
- Make sure the embryo comes off the device and floats into the medium
- Transfer the embryos through the warming media steps 2, 3 and 4
- From Step 4, transfer the embryo to either Holding/Transfer Medium or to IVC Medium

We are here to help – get in touch!

Remember:

“Luck never comes to the unprepared!” ☺