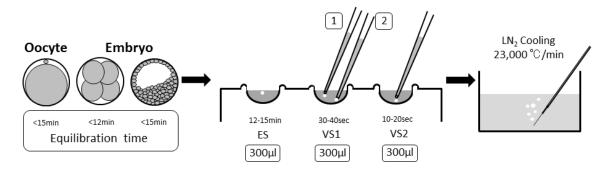


# For Oocytes and Embryos

# VITRIFICATION SOLUTION SET (110): For 10 times Uses



#### **Contents of Vitrification solutions set**

- -Equilibration Solution (ES): 2 vials of 1.6ml.
- -Vitrification Solution (VS): 4 vials of 1.6ml.

### Instructions:

### Preparation

- -The whole process should be performed under room temperature (25-27ºC).
- -Fill a nitrogen container.
- -Compare the thickness of the zona pellucida with the periviteline space, and take note for oocyte.
- -Important: Use a Pasteur pipet with the right diameter for oocyte, embryo (140-150  $\mu$ m) and Blastocyst (160-200  $\mu$ m).

### Equilibration of oocytes and embryos

- 1. Fill the Vitri Plate with 300  $\mu$ l of ES in the 1 $^{\rm o}$  well, and 300  $\mu$ l of VS in the 2 $^{\rm o}$  and 3 $^{\rm o}$  well.
- 2. Put the oocyte/embryo on the surface of ES in the 1º well.
- The oocyte/embryo will sink and begin to shrink, and gradually returns to the original size (maximum 15 min for oocyte and blastocysts, and 12 min for other stages of embryos).

## Vitrification

**Attention:** The following steps must be made in no less than 25 sec and a maximum time of 90 sec.

- 4. Transfer the oocyte/embryo to the half depth of the 2° well with VS. (Not with minimum volume of ES at the first step) The oocyte/embryo immediately floats to the surface of VS while washed.
- 5. After washing the inside wall of the pipette with fresh VS media, take only the oocyte/embryo and transfer it to the bottom of the well. Wait until the oocyte/embryo floating stops in VS.
- 6. Transfer the oocyte/embryo to the middle depth of the 3° well with VS, and mix the media by pipette around for 5 times.
- 7. Take only the oocyte/embryo at the top end in the pipette, and put it on the end of the cryotec seat with minimum volume of VS.
- 8. Immediately submerge the Cryotech into liquid nitrogen.
- 9. Place the cap, and store it in a nitrogen tank.

## Please stay Cryotec in liquid nitrogen at all times.

### **Quality Control Tests:**

This Lot Nº JIHA0115 (All Solutions)

Successfully passed the following controls:

- · Sterility: Sterility test.
- · Endotoxin by ES methodology (Each component).
- Efficiency: survival of 50/50 Mouse embryos and Porcine oocytes.

### Storage and stability

Solutions and kits can be transported under the room temperature, and then must be keep in the fridge at 2-8°C until the expiration date.

### Composition

- -Modified HEPES Buffered MEM
- -Hydroxy Propyl Cellulose
- -Ethylene Glycol
- -Dimethyl Sulfoxide
- -Endotoxin free Trehalose

## References

- Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: The CryoTop method. Theriogenology 67, 73-80, 2007
- Cobo A, Kuwayama M. Comparison of concomitant outcome schieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method.
  Fertil Steril. J89(6): 1657-64, 2007.
- Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reproductive BioMedicine Online 14, 5-667, 2007.
- Vajta G, Kuwayama M. Improving cryopreservation systems. Theriogenology 65(1), 236-44, 2006.
- Kuwayama M. Highly efficient vitrification method for cryopreservation of human oocytes. Reproductive BioMedicine Online 11:300-308, 2005.
- Ushijima J, Kuwayama M. High survival rate of bovine oocytes matured in vitro following vitrification. J Reprod Dev. 50:685-96, 2004.
- Fukui Y, Kuwayama M. Effect of cryodevice type and donor's sexal maturity on vitrification of minke whale oocytes at germinal vesicle stage. Zygote 12, 333-338, 2004.
- Hochi S, Kuwayama M. Improved Survival of Vitrified in vivo-derived porcine embryos. J. Reprod. Develop. 50, 481-486, 2004.
- Esaki R, Kuwayama M. Cryopreservation of porcine embryos derived from in vitri- matured oocytes. Biology of Reproduction. 71, 432-437, 2004.

Product for in vitro use only.

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