



For Oocytes and Embryos

Vitrification Kit (101) Instructions for Users

Contents:

- 1 vial of 1.0ml Equilibration Solution (ES).
- 2 vials of 1.0ml Vitrification Solution (VS).
- 4 Cryotecs:
 - 1 Cryotec can load more than one embryo.
 - Maximum load on Cryotec: five oocytes and five embryos.
- 3 Vitrif Plates with 3 wells

Composition:

This vitrification solution consists of 2 kinds of solutions. These solutions contain Gentamicin as an antimicrobial agent.
Vitrif Step1: Equilibration Solution (ES) contains ethylene glycol and DMSO as a cryoprotectant.
Vitrif Step2: Vitrification Solution (VS) contains ethylene glycol and DMSO as a cryoprotectant.
As a guide, this Kit is for up to 3 vitrification processes. However, once the vial is opened, the solution cannot be used.

Indications for use: The Kit is used to vitrify the human oocytes and embryos.

Attention during Storage:

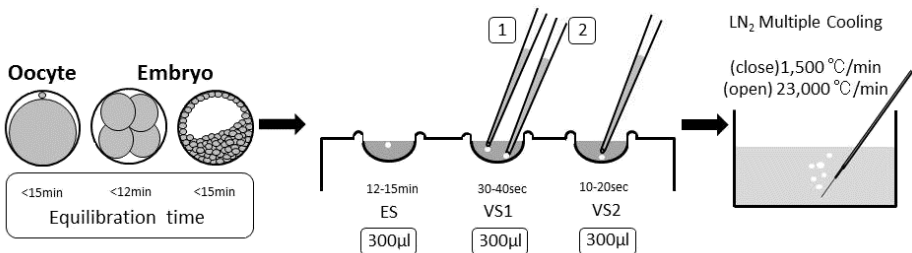
Solutions must be stored in the refrigerator at 2-8°C and Plates/Cryotecs must be stored at 10-25°C until the expiration date; these products can be used under the room temperature. Use before the expiration date indicated on the each of the labels.

Preparation

- The whole process should be performed under room temperature (25-27°C).
- Leave ES and VS under room temperature for at least 1 hour before vitrification.
- Do not use an electronic oven to warm up to room temperature instead of incubator.
- For reference, read the user manual carefully before use and follow the instructions.
- Fill a container with LN₂.
- Compare the thickness of the zona pellucida with the perivitelline space, and take note for embryos.
- Use an appropriate diameter size Pasteur pipette for oocytes/embryos (140-150 µm) and blastocysts (160-200 µm).

Instructions:

1. Fill the 1st well of a Vitrif Plate with 300 µl of ES, and both 2nd and 3rd wells with 300 µl of VS.
2. Put an oocyte/embryo on the surface of ES in the 1st well.
3. The oocyte/embryo will sink and begin to shrink, and then gradually return to the original size (maximum time is 15 min for oocytes and blastocysts, and 12 min for embryos at other stages).
4. Transfer the oocyte/embryo from the 1st well to the half depth of the 2nd well. The oocyte/embryo immediately floats to the surface of VS while being washed.
5. After washing the inside of the pipette with fresh VS media, aspirate and transfer the oocyte/embryo to the bottom of the well. Wait until the oocyte/embryo stops floating in VS.
6. Transfer the oocyte/embryo to the half depth of the 3rd well, and stir the media by pipette about 5 times.
7. Aspirate the oocyte/embryo to the tip of the pipette, and put it on the end of thin film attached to the Cryotec seat with a minimum (0.01 to 0.1µl) volume of VS.
For open system, Submerge the Cryotec into liquid nitrogen directly and then cover the Cryotec with cap.
For closed system, cover the Cryotec with cap and heat seal the Cryotec using heated tweezers to achieve the closed system, then immerse the capped Cryotec into liquid nitrogen.
8. Store the Cryotec in a LN₂-filled tank.





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Specification:

- Sterility: Irradiation (SAL10⁻⁶), Filtration (SAL10⁻³)
- Endotoxin: < 0.5EU/device (LAL) for Cryotec/Plate and < 0.6EU/ml (LAL) for solution.
- Cell MEA \geq 80% developed to blastocyst within 96hrs.
- Cooling rate: 1,500°C/min (Closed)
23,000°C/min (Open)

Caution:

This product is single use only. When this product is reused without discarding, there is a possibility of contamination. It is prohibited to reuse or re-sterilize.

Discard the residual solution, and do not reuse it.

If the valid date is expired, discard it immediately.

This product must be operated by fully qualified experts. Otherwise, do not use it.

Do not use if the product is exposed to a contaminated material or becomes turbid.

Do not use this product except the intended use.

Do not modify this product in any cases.

Do not use the product if the sterile packaging is damaged or if the product is damaged or defective.

Avoid any damage caused by heavy physical impact or heavyweight objects.

Opening and use of this product with aseptic technique is strongly recommended due to risk of contamination.

Discard this product in an appropriate manner in accordance with the regulations of your local authority.

Combined use with Plate and Cryotec is recommended.

The results of each lot are described in Certificate of Analysis and available upon request.

Product only for *in vitro* use.

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Product URL: <http://cryotech-japan.jp>

Symbols

No	Symbol	Description	No	Symbol	Description	No	Symbol	Description
1		'Manufacturer'	6		'Temperature limitation'	11		'Don't use if unit package is opened or damaged'
2		'Product code'	7		'Keep away from sunlight'	12		'Irradiation Sterilization'
3		'Batch code'	8		'Keep dry'	13		'Filtration Sterilization'
4		'Manufacturer date'	9		'Do not reuse'	14		'Consult instructions for use'
5		'Use by'	10		'Do not resterilize'	15		

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