

## For Oocytes and Embryos

# Warming Solution Set (205) Instructions for Users

### Contents:

- 5 vials of 1.8 ml Warming Solution (TS)
- 1 vial of 1.8 ml Diluent Solution (DS)
- 2 vials of 1.8 ml Washing Solution (WS)

### Composition:

This warming solution consists of 3 kinds of solutions. These solutions contain Gentamicin as antimicrobial agent.

Warm Step1: Warming Solution (TS) contains torehalose as thawing agent.

Warm Step2: Diluent Solution (DS) contains torehalose as thawing agent.

Warm Step3: Washing Solution (WS) does not contain torehalose as thawing agent.

As a guide, this Set is for up to 5 warming processes. However, once the vial is opened, the solution cannot be reused.

**Indications for use:** The solution is used to warm the vitrified oocytes and embryos.

### Attention during storage:

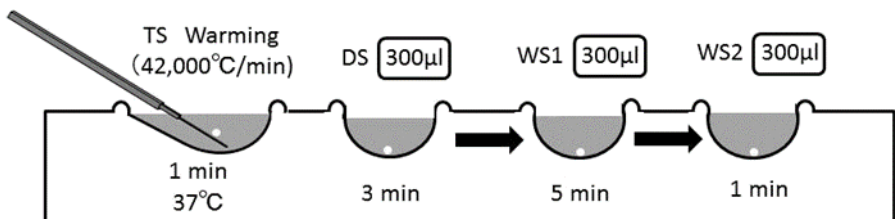
Solutions must be kept in the refrigerator at 2-8°C until the expiration date; these products can be used under the room temperature. Use before the expiration date indicated on the each of the labels.

### Preparation

- The whole process should be performed under room temperature (25-27°C).
- Use an appropriate diameter size Pasteur pipette for oocytes/embryos (140-150 μm) and blastocysts (160-200 μm).
- Place a Warm plate and TS vial (with rid) in an incubator at 37°C 3 hours before the use (overnight warming is recommended).
- Leave DS and WS vials as they are in the room temperature at least 1 hour before the use.
- Pull a patient's cane out of a LN2-filled tank, and remove the cover cap and lean the Cryotech against the inner wall of the cooling rack filled with LN2.

### Instructions:

1. Take the Warm Plate out of the incubator and fill the 2nd well with 300 μl of DS.
2. Take TS vial (1.8 ml) out from the incubator and expel total amount to the 1st square well.
  - Pull the Cryotech out from the cooling rack and put it into the 1st square well within 1 sec, and wait for 1 min.
3. While warming, oocyte/embryo naturally separates from the sheet and starts to float.
4. Aspirate the oocyte/embryo into the pipette at first. Then, aspirate TS (3 mm in length) into it slowly.
5. Insert the tip of the pipette into the center bottom of DS-filled well and expel TS slowly to make a TS layer at the bottom of it.
  - Expel embryo prudently so that it is placed at the bottom of TS layer. Wait for 3 minutes.
6. While waiting, fill the 3rd (WS1) and 4th wells (WS2) with 300 μl of WS each.
7. Aspirate the oocyte/embryo into the pipette at first. Then, aspirate DS (3 mm in length) into it slowly.
8. Insert the tip of the pipette into the center bottom of WS1 and expel DS slowly to make a DS layer at the bottom of it. Expel oocyte/embryo prudently so that it is placed at the bottom of DS layer. Memorize the shape of oocyte/embryo and turn the light of stereoscopic microscope off. Wait for 5 minutes.
9. 5 minutes later, compare the actual oocyte/embryo shape with the memorized one. If shrunk oocyte/embryo returned to the original volume, it is judged as survival.
10. Aspirate the oocyte/embryo into the pipette.
11. Put the oocyte/embryo on the left half side surface of WS2.
12. After the oocyte/embryo naturally sunk into the bottom of WS2 well, aspirate it again and put it on the right half side surface of WS2.
13. When the oocyte/embryo sunk again and reached the bottom, it is considered that the washing is finished.
14. Transfer the oocyte/embryo and put it into the droplet of the culture medium up to that point when ICSI and ET are performed.
  - Then, the oocyte/embryo is cultured for recovery.





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### Specification:

- Sterility: Filtration (SAL10<sup>-3</sup>)
- Endotoxin < 0.5EU/ml (LAL)
- Cell MEA ≥80% developed to blastocyst within 96hrs.
- Warming rate: 42,000°C/min

### 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 solutions and do not reuse them.

If the valid date is expired, discard the product 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 this product if the sterile packaging is damaged or 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.

3-hour culture for embryos is required.

**Product only for *in vitro* use.**

**Manufacturer: REPROLIFE Inc.**

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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		'Filtered Sterilization'
3		'Batch code'	8		'Keep dry'	13		'Consult instructions for use'
4		'Manufacturer date'	9		'Do not reuse'			
5		'Use by'	10		'Do not resterilize'			

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