

■Vitrification Cryotop Method for Oocyte and Embryo Thawing Media

March 26, 2021 Ver.4

Code: VT602US

Intended Use

Thawing Media –Thawing Media is indicated for use in the preparation and thawing of vitrified oocytes (MII), pronuclear (PN) zygotes through day 3 cleavage stage embryos, and blastocyst stage embryos.

CAUTION: Device is sterile if the package is unopened or undamaged. Do not use if package is broken.

CAUTION: Federal law restricts this device to sale by or on the order of

Thawing Kit

No.1 Thawing Solution (TS): 2 x 4ml
No.2 Diluent Solution (DS): 1 x 4ml
No.3 Washing Solution (WS): 1 x 4ml

Materials Required but Not Included:

Below required products are supplied by Kitazato.

- 35mm for TS
- Repro Plate with 6 wells or 2 Repro Plates with 3 wells

Instructions for Use (IFU)

Preparation

- Warm TS vial (sealed) and a Petri Dish in an incubator to 37°C.(>1.5hrs) Pour the full content of TS into the Petri Dish.
- Bring DS and WS to room temperature (25-27 °C as recommendation). All the procedure should be performed at room temperature.
- Drop 300µl each of DS, WS1 and WS2 on the Repro Plate with micro pipette.

NOTE: Use pasteur pipette that has a suitable internal diameter for Oocytes (External diameter: 120µm) or Embryos (External diameter: 120µm-250µm).

Thawing

1. Retrieve the cane which has the specific Cryotop and quickly immerse the cane in a container with fresh liquid nitrogen. Collect the specific Cryotop from the cane and return the cane to the storage tank. Check the information about the patient on the handle/cap of the Cryotop.

CAUTION: The Cryotop except its handle must remain immersed in liquid nitrogen at all time.

2. Prepare the liquid nitrogen container with selected Cryotop devices for thawing near the stereo microscope.
3. Remove the Cryotop from the protective straw cap taking care to avoid touching the tip holding Oocyte or Embryo with the wall of the straw cap.

CAUTION: Make sure the tip of the Cryotop do not touch liquid nitrogen.

4. Quickly immerse the tip of the Cryotop holding Oocyte or Embryo into the TS. This procedure should be completed within one second. Leave it for 1 minute.
5. Aspirate the Oocyte or Embryo with the pasteur pipette and gently place it on the BOTTOM of the DS. Leave it for 3minutes.
6. Aspirate the Oocyte or Embryo with the pasteur pipette and gently place it on the BOTTOM of the WS1. Leave it for 5minutes.
7. Aspirate the Oocyte or Embryo with the pasteur pipette and gently place it on the TOP of the WS2. After the Oocyte or Embryo drops to the bottom of the WS2, repeat this process again in WS2.
8. Transfer the Oocyte or Embryo to a culture dish containing the appropriate culture medium. Incubate the Oocyte or Embryo in a 37°C incubator to complete recovery.

NOTE: Incubation of oocytes for 2 hours, embryos for 3 hours is ideal for complete recovery.

Quality Control Specification

Each lot of Thawing media receives the following tests:

- Sterility by the current USP Sterility Test <71>
- Endotoxin by LAL methodology <0.25EU/mL (LAL)
- Mouse Embryo Assay (1-cell to Blastocyst at 96h:≥80%)
- pH (USP)
- Osmolality (USP)

Storage instructions and stability

Store the vials at 2-8 °C

Composition

- HEPES within Basic Culture Medium
- Trehalose
- Hydroxypropyl Cellulose
- Gentamicin

Warning

- Do not re-sterilize.
- Do not use solution that shows cloudiness or turned yellow for solution with phenol red.
- Do not use if sterile packaging is broken.
- Media must be stored in original unopened container, refrigerated at 2-8°C.
- To avoid contamination, do not reuse.

Cautions

- Read the instructions for use prior to use.
- This product is intended to be used by only medical specialist of fertility treatment.
- Aseptic technique should be used.
- Use sterilized equipment, materials and items.
- Decontaminate the workroom.
- Follow procedures in an environmentally controlled room.
- The long term safety of thawing procedure on oocytes or embryos is unknown.
- The Thawing media contains the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.

References

1. Cobo A., Obstetric and perinatal outcome of babies born from vitrified oocytes. Fertility and Sterility, 2014.
2. Rienzi L., Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. Human Reproduction, 2012.
3. Shi W., Perinatal and neonatal outcomes of 494 babies delivered from 972 vitrified embryo transfers. Fertility and Sterility, 2012.
4. Cobo A., Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertility and Sterility, 2012.
5. Trokudes KM., Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program. Fertility & Sterility, 2011.
6. Inoue F., Hydroxypropyl cellulose as a macromolecular supplement for cryopreservation by vitrification of bovine oocytes and blastocysts and human oocytes. ESHRE and ASRM, 2011.

KITAZATO[®]

Copyright (C) Kitazato Corporation All Rights Reserved.



Kitazato Corporation

100-10 Yanagishima, Fuji, Shizuoka 416-0932 Japan
TEL: +81-545-65-7122 FAX: +81-545-65-7128