■Vitrification Cryotop Method for Oocyte and Embryo

Vitrification Media

Code: VT601US

March 26, 2021 Ver.4

Intended Use

The Vitrification Media – Vitrification media is indicated for use in the preparation, vitrification and storage of 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 damaged.

CAUTION: Federal law restricts this device to sale by or on the order of a physician or practitioner trained in its use.

Vitrification Kit

No.0 Basic Solution (BS): 1 x 1.5ml
No.1 Equilibration Solution (ES): 1 x 1.5ml
No.2 Vitrification Solution (VS): 2 x 1.5ml

Materials Required but Not Included:

Below required products are supplied by Kitazato.

- CryotopSC/CL/US: 1 Cryotop stores up to 4 Oocytes or 4 Embryos as a recommendation.
- Repro Plates with 6 wells or 2 Repro Plates with 3 wells

Instructions for Use (IFU)

Preparation

- Fill 90% of Cooling Rack with fresh liquid nitrogen.
- Open the sterile pack of Cryotop and write necessary information about the patient on the handle/cap of Cryotop.
- Take off the cap of Cryotop.
- Compare the width of perivitelline space with the thickness of zona pellucida and record it.
- Bring BS, ES and VS to room temperature (25-27°C). All the procedures should be performed in room temperature.

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

Oocyte Equilibration

1. Drop 20μl of BS and 300μl each of VS1 and VS2 to each well on the Repro Plate with micro pipette.
2. Transfer the oocyte from the culture dish to the BOTTOM of BS.
3. Step 1. Add ES 20μl gently to the SURFACE of BS and leave it for 3 minutes.
   Step 2. Add another ES 20μl gently to the SURFACE of BS and leave it for 3 minutes.
   Step 3. Add another ES 240μl gently to the SURFACE of BS and leave it for 9 minutes

Embryo Equilibration

1. Drop 300μl of ES, VS1 and VS2 on the Repro plate using micro pipette.
2. Transfer the Embryo to the SURFACE center of ES.
3. While the embryo free-falls, it spontaneously begins to shrink and then gradually returns to its original size by ES infiltration (within 15 minutes)

Vitrification

The following steps from 1 to 5 should be completed between 60 and 90 seconds.

1. Aspirate the Oocyte/Embryo from ES at the tip of pasteur pipette. Transfer the Oocyte/Embryo to the SURFACE center of VS1.
2. Aspirate the Oocyte/Embryo with pasteur pipette and blow it out to VS1. Repeat this work 3 times changing the position in VS1.
3. Transfer the Oocyte or Embryo to VS2. Move the Oocyte/Embryo changing positions twice in VS2 with pasteur pipette.
4. After vitrification procedures, place the Cryotop under the microscope and adjust the focus on the black mark of the Cryotop. The black mark should be face-up.
5. Gently place Oocytes/Embryos on the tip of the Cryotop sheet with minimal volume (≤0.1μl) of vitrification media using a suitable pipette.

CAUTION: A larger volume may raise the risk of reducing cooling rate.

NOTE: The recommended load of the Cryotop is up to 4 embryos/oocytes.

6. CryotopSC/CL: Place the Aluminum Block in Cooling Rack and fill 90% of Cooling Rack with fresh liquid nitrogen; allow the boiling to finish. (about 5 minutes). Place the straw cap in the Aluminum Block and cut the upper mark band on the straw cap using the Straw Cutter.
7. CryotopSC/CL: Hold the straw cap with tweezers and insert the Cryotop, making sure the tip do not touch the cap, holding up the CryotopSC/CL sheet within the 2.5cm height from the surface of liquid nitrogen. Seal the upper part of the straw cap with the sealer.
   CryotopUS: Hold the straw cap with tweezers and insert the CryotopUS, making sure the tip do not touch the cap. Fit the CryotopUS with the cap by hands tightly.
8. Put the Cryotop in a cane and store in tank for long term storage.

CAUTION: Take care that the Cryotop remains immersed in liquid nitrogen at all times until thawing.

Quality Control Specification

Each lot of **Vitrification 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 ºC

Composition

- HEPES within Basic Culture Medium
- Ethylene Glycol
- Dimethyl Sulfoxide
- 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 2-8ºC.
- 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 vitrification procedures on oocytes or embryos is unknown.
- The Vitrification 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 ooytes and blastocysts and human oocytes. ESHRE and ASRM, 2011.

KITAZATO®

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