



Vitrification Media



Intended use

This product is to be used for vitrification of oocytes (MII) and embryos.

Vitrification media

- No. 0 (colour code: white) Basic Solution (BS): 1×1.5 ml vial (only for oocyte (MII) vitrification)
! Caution: Before use, check the specifications of the container and labeling (number marked on top of the cap, cap colour, vial labeling colour, solution name, and volume). If you notice anything unusual regarding the items mentioned above, do not use the product and please contact the distributor

Recommended equipment

- Cryotop: 1 Cryotop stores up to 4 oocytes (MII) or 4 Embryos as a recommendation.
- ReproPlate: with 6 wells
- Cooling Rack
! Caution: This product is sterilized. Please handle the product in a sterile field with a clean bench.

Instructions for use

Preparation

- Fill 90% of the Cooling Rack with fresh liquid nitrogen.
- Compare the width of perivitelline space with the thickness of zona pellucida and record it.
- Bring BS, ES and VS to room temperature (23-27 °C as recommendation).
- Use a sterilised pipette as a handling tool, with a suitable internal diameter for oocytes or embryos.
- The recommended internal diameters are as follows: 120 µm for oocytes (MII), 120 µm for pronuclear stage embryos, 150 to 180 µm for cleavage stage embryos, and 180 to 250 µm for blastocyst stage embryos.

Equilibration

! Caution: Equilibration procedures for Oocyte (MII) and Embryo are different.

Equilibration of oocytes (MII)

1. Using a pipette place a 20 µl of BS into the first well and 300 µl of VS in both the second and third wells of the Reproplate.
2. Step 0: Transfer the Oocyte (MII) from the culture dish to the BOTTOM of BS.
3. Step 1: Immediately add ES 20 µl gently on the TOP of BS of the first well and leave it for 3 minutes.
 Add another ES 20 µl gently on the TOP of the first well and leave it for 3 minutes.
 Add another ES 240 µl gently on the TOP of the first well and leave it for 9 minutes.

Equilibration of embryos

1. Drop each 300 µl of ES into first well, VS into second and third well on the Repro plate using pipette.
2. Step 1: Transfer the Embryo to the TOP center of ES from the culture dish. It will spontaneously begin to shrink and then gradually return to its original size by absorbing the ES solution (within 15 minutes).

Vitrification

! Caution: The following steps from 1 to 9 should be completed between 60 and 90 seconds.

1. Aspirate the oocyte (MII)/embryo from ES with the tip of a pipette.
2. Step 2: Transfer the oocyte (MII)/embryo to the TOP centre of the VS of second well.
3. Aspirate the oocyte (MII) / embryo with a pipette and blow it out. Repeat this process 3 times, changing the position in the VS of second well.
4. Transfer the oocyte (MII)/embryo to the VS of third well.

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5. Change the position of the oocyte (MII)/embryo in the VS of third well with a pipette.
6. Place the oocyte (MII)/embryo by the black line on the Cryotop.
7. Make a planar droplet.
8. Make sure if the oocyte (MII)/embryo is on the Cryotop with a minimal volume of the VS of third well (less than 0.1µl) under a microscope.
9. Plunge the Cryotop immediately in liquid nitrogen.
10. Put the Cryotop in a cane and store it in a storage tank.

Quality control specification

The following tests were performed for each lot of this product:

- Sterility by the Sterility Test (EP)
- Endotoxin by LAL methodology
- Mouse Embryo Assay (One Cell)
- pH (EP)
- Osmolality (EP)

Storage instructions and stability

Store the vials at 2 to 8 °C.

This product is stable until the expiry date labeled on the vial.

Composition

- HEPES within Basic Culture Medium
- Ethylene Glycol
- Dimethyl Sulfoxide
- Trehalose
- Hydroxypropyl Cellulose
- Gentamicin

Contraindications

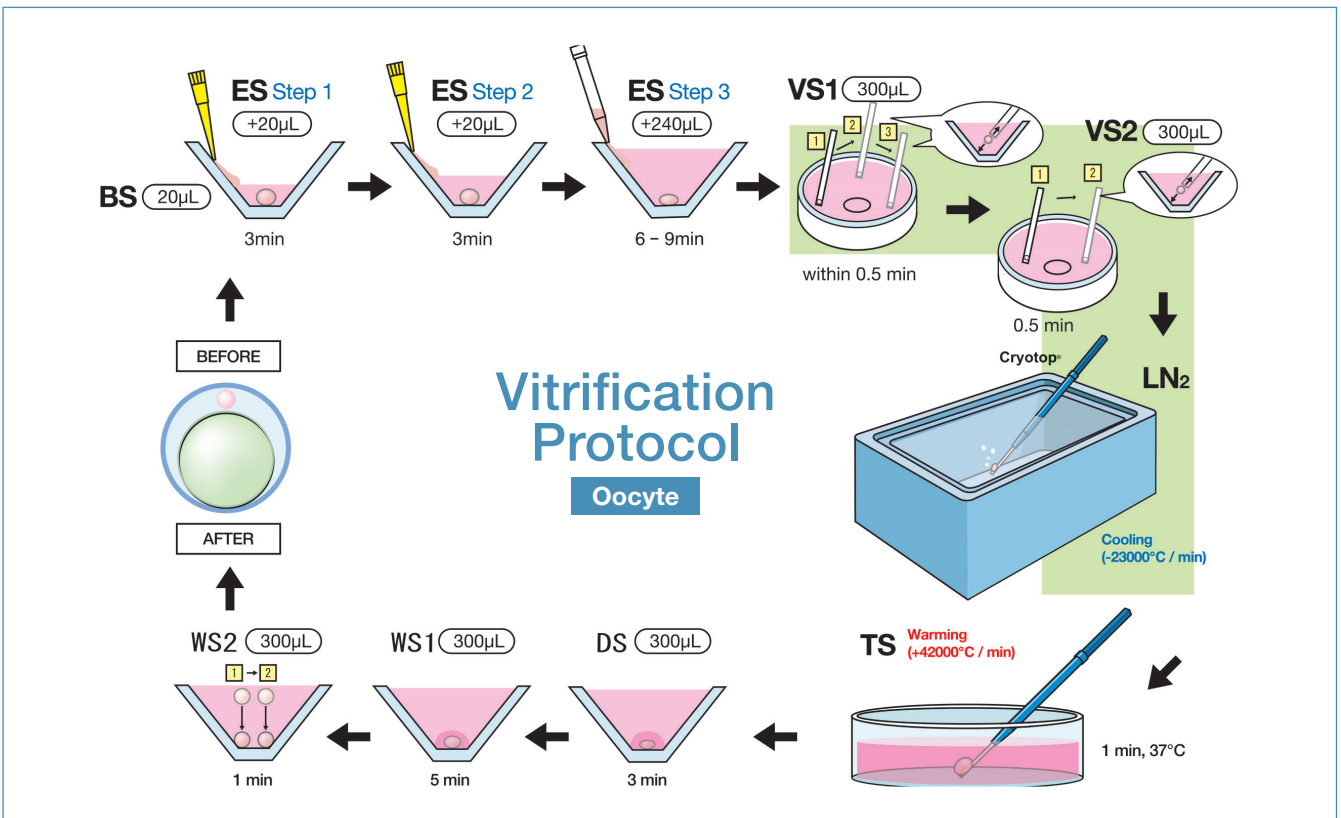
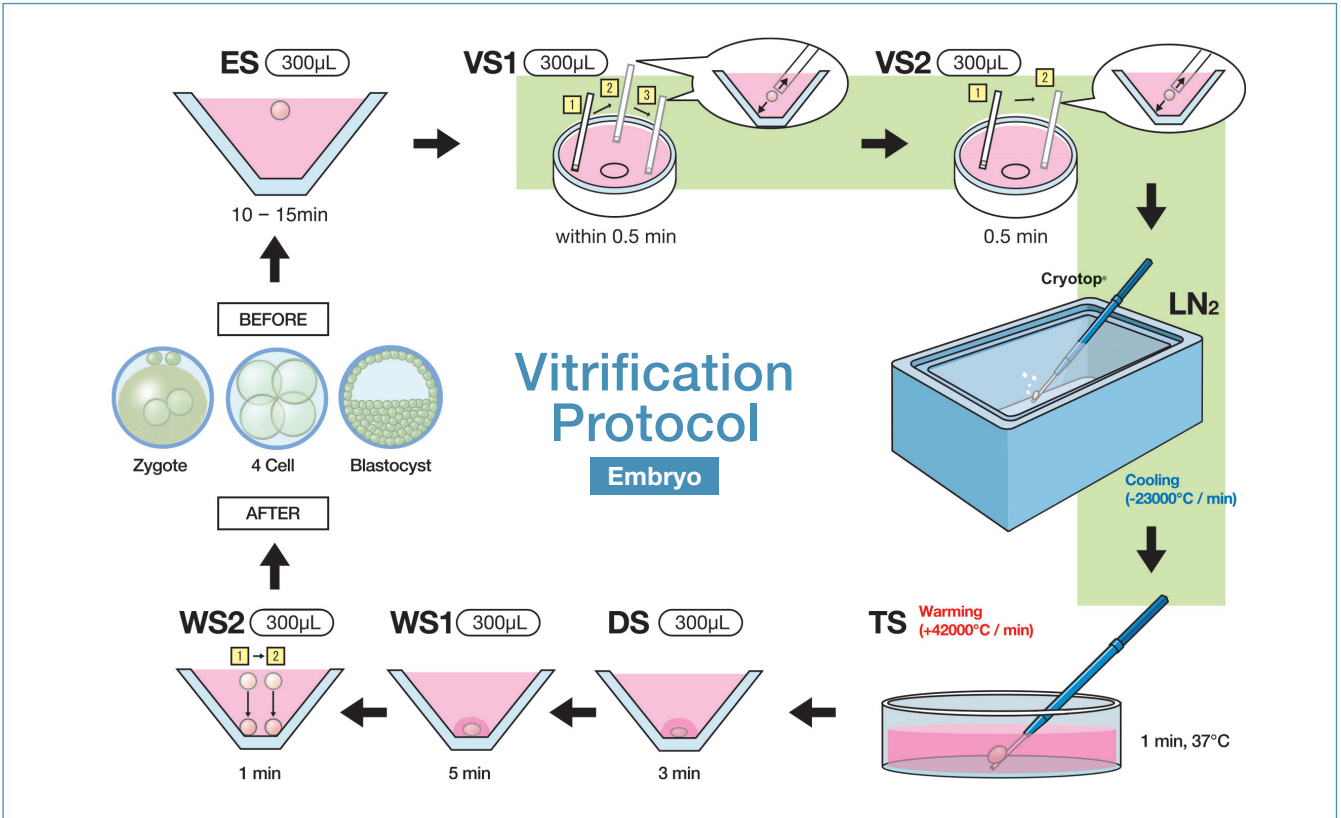
- Do not re-sterilise.
- Do not re-use. Re-use may cause contamination.
- Do not use solution that shows cloudiness or becomes discoloured.
- Do not use the product if you notice anything unusual regarding the specifications on the label (number, colour, name, volume).
- Device is sterilised if the package or container is unopened or undamaged. Do not use if the package or container are opened or damaged.
- Upon delivery media must be stored in original unopened container and refrigerated at 2-8 °C.
- Do not use and please discard if the media is not stored under refrigeration (2 to 8 °C).
- Do not use the product if past the expiration date.

Warning

- Read the instructions for use prior to use.
- Use the KITAZATO thawing media to thaw the oocytes (MII) or embryos vitrified with KITAZATO vitrification media
- This product is intended to be used by medical specialists trained in fertility treatment.
- Aseptic technique should be used.
- Use sterilised equipment and materials only.
- In case of eye or skin contact with Vitrification/Thawing media, immediately flush eye/skin with water.
- Morphologically abnormal oocytes, embryos, or significantly poor grade oocytes or embryos are unsuitable for cryopreservation.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of infection, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- Vitrification media contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitised to this antibiotic.

Note: The long-term safety of the vitrification technique and maximum storage in liquid nitrogen has not been established and is unknown.

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