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VITRIFICATION MEDIA

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EN-VITRIFICATION MEDIA

GENERAL INFORMATION Basic UDI-DI: 45822314vTUN This Instruction for Use covers: VT601, Tarot composition: Patient undergoing ART procedures For professional use only **INTENDED USE** This product is to be used for vitrification of oocytes (MII) and embryos (1 cell to blastocyst stage) **specimens CONTENT** - No. 0 (Color: clear) Basic Solution (BS): 1x1.5 ml vial (only for oocyte (MII) vitrification) - No. 1 (Color: blue) Equilibration Solution (ES): 1x1.5 ml vial - No. 2 (Color: green) Vitrification (VS): 2x1.5 ml vials. **Caution:** Before use, check the specimen and solutions for damage. Do not use if damaged. Do not use the product if the cap, cap color, vial labelling, color code, or tear seal is damaged. If you notice anything unusual regarding the items mentioned above, don't use the product and contact the manufacturer. **Caution:** This product is sterilized. Handle it under aseptic conditions in a clean bench. **RECOMMENDED EQUIPMENT** Incubator, Stereomicroscope, Warming plate, Cryotop holder, Cryotop. Use devices intended for its particular use. We recommend the use of KITAZATO products in combination with the device. **COMPOSITION** • HEPES within Basic Media • Propanediol (1,2-propanediol) • Dimethyl Sulfoxide • Trehalose • Hydroxypropyl Cellulose • Gentamicin (VT601X) **PRODUCT SPECIFICATIONS** • Sterility: SAL 10-6 • Endotoxin by LAL methodology • 1cell MEA (blastocysts after 96h) ≥ 80% • pH (RT) • Osmolality (RT) Analysis certificate and MSDS are available upon request and can be downloaded from our website (https://kitazato-vf.com) **INSTRUCTIONS FOR USE Preparation:** • Fill 90 % of the Cooling Rack with fresh liquid nitrogen. Drop BS, ES and VS into the liquid nitrogen. **Caution:** Use only Cryotop® Holder, which is a suitable internal diameter for oocytes or embryos. The recommended internal diameters are: 120 µm for oocytes (MII), 120 µm for pronuclear stage embryos, 150 µm for 2 cell to morula stage embryos, and 180 µm for 3 cell to blastocyst stage. **A. Vitrification procedure using Repro Plate** Equilibration **Caution:** Equilibration procedures for Oocytes (MII) and Embryos are different. **Equilibration of oocytes (MII)** 1. Drop 0.5 ml of BS into the Repro Plate well (Blue). 2. Place the oocyte(s) in the BS well and leave it for 30 minutes. Add another 0.5 ml of BS into the Repro Plate with pipette. 2. Transfer the Oocyte (MII) from the culture dish to the BOTTOM of BS. 3. Immediately add ES 20 µl gently on the TOP of BS in 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 3 minutes. Add another ES 240 µl gently on the TOP of the first well and leave it for 6~9 minutes. **Equilibration of embryos:** 1. Dispense 30 µl, of ES into the first well of the Repro Plate using pipette. 2. Place the embryo(s) in BS to the TOP centre of ES from the culture dish. It will sink and begin to shrink. Then it will gradually return to its original size by absorbing the ES solution. 3. Wait for 6 min. 4. Add 240 µl of ES on the TOP of the BS droplet. **B. Vitrification: Caution:** Complete steps from 1 to 9 between 60 and 90 seconds. 1. Dispense 300 µL of VS into second and third well on the Repro Plate with a pipette. 2. Aspirate the specimen from 1 to 3 times in the ES droplet. 3. Place the specimen in the TOP centre of the VS1 of second well. 4. Aspirate the specimen with a pipette and blow it out repeat this process 3 times. Aspirate the specimen in the VS1 of second well. 5. Transfer the specimen to the BOTTOM of VS2 on the third well with a pipette. 6. Change the position of the specimen in the VS2 of third well with a pipette 7. Place the specimen on the top stack on the Cryotop® holder. 8. Place the top stack on the Cryotop® men is on the Cryotop® with a minimal volume of the VS (less than 0.1 µL) of third well under a microscope. 9. Plunge the Cryotop immediately in liquid nitrogen and shake it vigorously. 10. Insert the Cryotop® in the outer straw and seal it. **Warming** 1. Prepare 300 µl of VS in the first well of the Repro Plate. 2. Place the Cryotop® men is on the Cryotop® with a minimal volume of the VS (less than 0.1 µL) of third well under a microscope. 9. Plunge the Cryotop immediately in liquid nitrogen and shake it vigorously. 7. Insert the Cryotop® in the outer straw and seal it. 8. Immerse the Cryotop® in the warm solution (37°C) for 1 min. 9. Transfer the Cryotop® to the ES droplet. 10. Place the Cryotop® under a microscope. 6. Plunge the Cryotop®

| Unique Device Identifier (UDI) | Brand name | Catalogue number | Catalogue number | Catalogue number