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SAGE™ Vitrification Warming Kit

Product No.

ART-8030

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Indication for use
SAGE Vitrification Warming Kit is intended for use in the thawing of vitrified oocytes (MII), pronuclear (PN) zygotes through day 3 cleavage stage embryos and blastocyst stage embryos.

Product description
1.0 M Sucrose Warming Solution (ART-8030-A) is a MOPS buffered solution of modified HTF containing non-essential and essential amino acids, gentamicin sulfate (10 µg/mL), 1.0 M sucrose and 12 mg/mL human albumin.
0.5 M Sucrose Warming Solution (ART-8030-B) is a MOPS buffered solution of modified HTF containing non-essential and essential amino acids, gentamicin sulfate (10 µg/mL), 0.5 M sucrose and 12 mg/mL human albumin.
MOPS Solution (ART-8030-C) is a MOPS buffered solution of modified HTF containing non-essential and essential amino acids, gentamicin sulfate (10 µg/mL) and 12 mg/mL human albumin.

Package
ART-8030-A: 1.0 M Sucrose Warming Solution (1M WS)
ART-8030-B: 0.5 M Sucrose Warming Solution (0.5M WS)
ART-8030-C: MOPS Solution (MS)

Pack size
ART-8030:
ART-8030-A: 1 x 4 mL vial
ART-8030-B: 1 x 2 mL vial
ART-8030-C: 1 x 6 mL vial

Contains
Human Serum Albumin 12 mg/mL
Gentamicin sulphate 10 µg/mL

Quality Assurance
Sterility tested (Ph.Eur., USP<71>)
Osmolality tested (Ph.Eur., USP<785>)
pH tested (pH.Eur., USP<791>)
Endotoxin tested <0.5 EU/mL (Ph.Eur., USP<85>)
1-cell MEA ≥80% blastocyst at 96h
HSA analysis (Ph.Eur., USP).
Note: The results of each batch are stated on a Certificate of Analysis, which is available on www.origio.com.

STORAGE INSTRUCTIONS AND STABILITY
The product is aseptically processed and supplied sterile.
Store in original container at 2°C - 8°C, protected from light.
Do not freeze.
Discard excess (unused) media following warming.
The product is provided in vials intended for single use.
When stored as directed by the manufacturer the product is stable until the expiry date shown on the label.

PRECAUTIONS AND WARNINGS
Warning: The long-term safety of vitrification on children born from this procedure is unknown.
Warning: This media product includes the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.
Caution: Federal law restricts this device to sale by or on the order of a physician or trained in its use (Rx Only).
Caution: This product contains albumin, a derivative of human blood.
Caution: All blood products should be treated as potentially infectious. Source material from which this product was derived was tested and found non-reactive for HBsAg and negative for Anti-HIV-1/-2, HIV-1, HBV, and HCV. Furthermore, source material has been tested for parvovirus B19 and found to be non-elevated. No known test methods offer assurances that products derived from human blood will not transmit infectious agents.
Caution: Do not use if the product becomes discolored, cloudy, turbid, or shows any evidence of microbial contamination.
Caution: Do not use the product if packaging appears damaged or the expiry date has been exceeded.
Caution: The user should read and understand the Instruction for Use, Warnings and Precautions, and be trained in the correct procedure before using the Vitrification and Vitrification Warming Kits for vitrification procedures.

WARMING AND DILUTION PROTOCOL
The warming and dilution procedure is to be performed at 35-37°C. Use a heated microscope stage for the procedures below.
Minimize exposure of specimens to light during incubation in the Warming Solutions.
Bring the solutions to 35-37°C before use.
Refer to the directions for use accompanying the carrier device.

A. Procedure using micro-drop volumes of solutions – zygotes, embryos and blastocysts
(note: This product should not be used for oocytes – use instead the larger volume protocol below).
Maximum of 1 straw processed per dispensed media.

1. Fill the liquid nitrogen reservoir with liquid nitrogen to a sufficient depth to completely submerge a cryotube or goblet containing the carrier. Place near the liquid nitrogen storage tank containing the vitrified samples to be warmed.

2. Remove the cryocanes with the goblets containing the carrier with vitrified specimen(s) and quickly transfer them to the reservoir containing liquid nitrogen, keeping the carrier under liquid nitrogen at all times.

3. Follow the instructions that accompany the carrier being used.

4. Place the liquid nitrogen reservoir close to the microscope for rapid manipulation.

5. Label the dish(es) with the necessary information and pre-warm to 37°C

6. Make sure the contents of each vial of 1M Sucrose Warming Solution (WS), 0.5M WS and MOPS Solution (MS) are well-mixed by gentle inversion several times before use.

7. Prepare the dish(es) by aseptically dispensing 0.5-4 mL of 1M WS (dependent on carrier and warming process) and two 20 µL drops of 0.5M WS (see Fig 1). Three 20 µL drops of MS will be set up later at Step 12.

8. Using tweezers, locate the carrier on the cane in the liquid nitrogen reservoir.

9. Carefully remove the carrier from the cane, keeping the part containing the specimen(s) under the surface of the liquid nitrogen.

10. Follow the directions for use that accompany the carrier being used. Immediately (within 2 seconds) immerse the device after it has been extracted from its protective covering into the 1M WS. The specimen(s) will float from the device into the 1M WS. Leave the specimen(s) in this solution for one minute. They will remain shrunken and float to the top of the drop.
Note: After each transfer of specimen(s), blow out any remaining fluid in the transfer pipette and draw up some solution from the next drop prior to the next manipulation. Avoid creating air bubbles during transfers.

11. Draw up some 0.5M WS into the transfer pipette and transfer the specimen(s) from the drop of 1M WS with minimal volume to the bottom of the first drop of 0.5M WS and leave for 2 minutes.

12. Then transfer the specimen(s) to the bottom of the second drop of 0.5M WS and leave for for 2 minutes.
Note: The specimen(s) will remain shrunken during exposure to 0.5M WS. During this time, set up three 20 µL drops of MOPS Solution (MS; MS1, MS2, MS3), as shown in Figure 1.

13. Transfer the specimen(s) to the bottom of the first drop of MS (MS1) and leave for 3 minutes.

14. Then transfer the specimen(s) to the top of the second drop of MS (MS2) and leave for 3 minutes.

15. Transfer the specimen(s) to the top of the third drop of MS (MS3) and leave for 3 minutes.

16. Finally, transfer the specimen(s) to a dish of pre-equilibrated appropriate culture medium and place in a CO_2 incubator at 37°C for 3-4 hours to allow for recovery prior to further manipulations and/or transfer.

If more specimen(s) are to be warmed, repeat steps 6 to 16 above, using fresh drops of the warming solutions.

B. Procedure using larger volumes – oocytes, zygotes, embryos and blastocysts
Maximum of 1 straw processed per dispensed media.

1. Remove the cryocanes, with the goblets containing the carrier of vitrified specimen(s), and quickly transfer them to the reservoir containing liquid nitrogen, keeping the carrier under liquid nitrogen at all times.

2. Follow the instructions for use that accompany the carrier being used.

3. Place the liquid nitrogen reservoir close to the microscope for rapid manipulation.

4. Label the dish(es) with the necessary information and pre-warm to 37°C.

5. Make sure the contents of each vial of 1M WS, 0.5M WS and MS are well-mixed by gentle inversion several times before use.

6. Prepare the dish(es) by aseptically dispensing the following: 0.5-4 mL (dependent on the carrier and warming process) of 1M WS into Well 1, 1 mL of 0.5M WS into Well 2 and 1 mL of MS into each of Wells 3 and 4 (see Figure 2 below).

7. Using tweezers, locate the carrier on the cane in the liquid nitrogen reservoir.

8. Carefully remove the carrier from the cane, keeping the lower part containing the specimen(s) under the surface of the liquid nitrogen.

9. Follow the directions for use that accompany the carrier being used. Immediately (within 2 seconds) immerse the device, after it has been extracted from its protective covering, into well 1 containing 1M WS. The specimen(s) will float from the device into the 1M WS. Leave the specimen(s) in this solution for one minute. They will remain shrunken and float to the top of the well.
Note: After each transfer of oocytes, zygotes, embryos and blastocysts, blow out any remaining fluid in the transfer pipette and draw up some solution from the next drop prior to the next manipulation. Avoid creating air bubbles during transfers.

10. Draw up some 0.5M WS into the transfer pipette and transfer the specimen(s) from Well 1 to Well 2 containing 0.5M WS and leave for 3 minutes.
Note: The specimen(s) will remain shrunken during exposure to 0.5M WS.

11. Then transfer the specimen(s) to the top of Well 3 containing MS1 and leave for 5 minutes.

12. Next, transfer the specimen(s) to the top of Well 4 containing MS2 and leave for 5 minutes.

13. Finally, transfer the specimen(s) to a dish of pre-equilibrated appropriate culture medium and place in a CO_2 incubator at 37°C for 3-4 hours to allow for recovery prior to further manipulations and/or transfer.

If more specimen(s) are to be warmed, repeat steps 8 to 13 immediately using fresh drops of 1M WS, 0.5M WS and MS.

Laboratories should determine the particular details to use for each particular procedure.

Figure 1

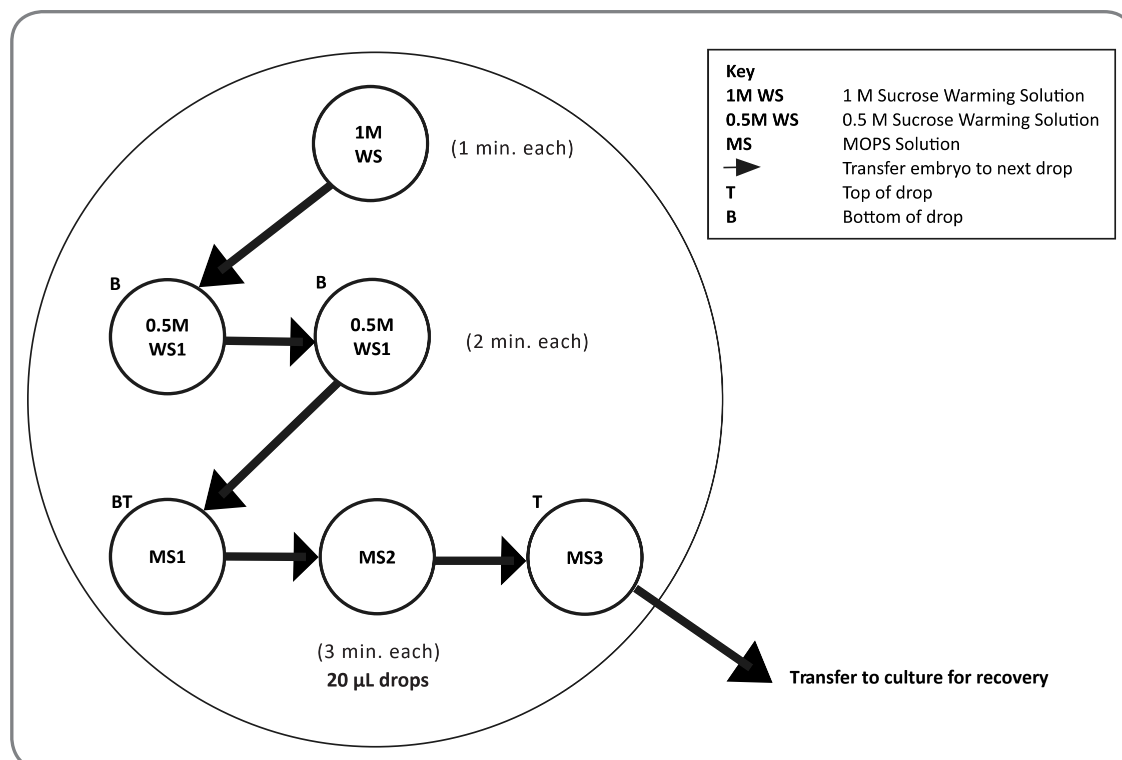

Figure 2