5854-02
Ver.02: 2018NOV23

SAGE™ Vitrification Kit

Product No.

ART-8025

Customer Service:
E-mail: customer.service@origio.com
Tel.: +45 46 79 02 02
Fax: +45 46 79 03 02

ORIGIO a/s
A CooperSurgical Company
Knardrupvej 2
DK-2760 Måløv
Denmark
www.origio.com
Tel.: +45 46790200
Fax: +45 46790300

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

PRODUCT DESCRIPTION
Equilibration Solution (ART-8025-A) is a MOPS buffered solution of modified HTF containing non-essential and essential amino acids, gentamicin sulfate (10 µg/mL), 7.5% (v/v) each of DMSO and ethylene glycol and 12 mg/mL human albumin.

Vitrification Solution (ART-8025-B) is a MOPS buffered solution of modified HTF containing non-essential and essential amino acids, gentamicin sulfate (10 µg/mL), 15% (v/v) each of DMSO and ethylene glycol, 12 mg/mL human albumin and 0.6 M sucrose.

Package
ART-8025-A: Equilibration Solution (ES)
ART-8025-B: Vitrification Solution (VS)

Pack size
ART-8025: 2 x 2 mL:
ART-8025-A: 1 x 2 mL
ART-8025-B: 1 x 2 mL

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.
Caution: Use a legally marketed storage device indicated for use in vitrification procedures for oocytes or the embryo stage to be vitrified. Use a closed storage system to prevent the potential risk of viral contamination and do not use open storage systems where the sample comes in direct contact with liquid nitrogen. The rate of cooling in the storage device should be between 12,000 to 23,000ºC/min.

VITRIFICATION PROTOCOL
The vitrification procedure is to be performed at room temperature (20-25°C). Bring the solutions to room temperature before use.

Caution:
• Do not use a heated microscope stage for the procedures listed below (A, B and C).
• Minimize exposure of specimens to light during incubation in Equilibration and Vitrification Solutions.

Suggested Equilibration Timings

Note: Optimal timings must be confirmed in individual laboratory conditions	
Oocytes and zygotes	10 to 12 min.
Embryos	5 to 7 min.
Blastocysts expanded	12 to 15 min.
Blastocysts collapsed	5 min.

A. Procedure using small volume drops of solutions (zygotes/embryos and blastocysts only)
Maximum of 4 zygotes/embryos/ blastocysts processed per dispensed media.

1. Fill the liquid nitrogen reservoir with liquid nitrogen and prepare system for storage of the vitrified specimens.

2. Label the dish(es) and carrier with the necessary information.

3. Make sure the contents of each vial of Equilibration Solution (ES) and Vitrification Solution (VS) are well mixed by gentle inversion several times before use.

4. Prepare a dish by aseptically dispensing a minimum 100 µL of ES (see Figure 1).

5. Remove the culture dish containing specimen(s) from the incubator and check their quality.

6. Carefully transfer the specimen(s) with a minimal volume of culture medium to the ES and start the timer. Allow the specimen(s) to equilibrate for 5 to 15 minutes. The specimen(s) will shrink and then gradually re-expand to their original size, indicating that equilibration is complete.

7. Towards the end of the equilibration time in ES, set up 4 x 20 µL drops of VS as shown in Figure 1.

8. Steps 9-13 should be completed within 90-110 seconds.

9. After equilibration in ES is complete, draw up some ES into the transfer pipette and transfer the specimen(s) with minimal volume from the ES into VS1 and leave for 5 seconds.

10. Quickly transfer the specimen(s) from VS1 to the center of the second drop of VS (VS2) and leave for 5 seconds.

11. Next, transfer the specimen(s) from VS2 to the center of the third drop of VS (VS3) and leave for 10 seconds.

12. Finally, transfer the specimen(s) from VS3 to the bottom of the fourth drop of VS (VS4). This step together with step 13 should be completed within 60-90 seconds.

13. For the vitrification procedure, carefully transfer the specimen(s) with minimal volume of VS from drop VS4 to the carrier, as recommended by the manufacturer.

If more specimens are to be vitrified, repeat steps 3 to 13 above using fresh solutions of ES and VS.

B. Procedure – Oocytes
Maximum of 2 oocytes processed per dispensed media.

1. Fill the liquid nitrogen reservoir with liquid nitrogen and prepare system for storage of the vitrified oocytes.

2. Label the dish(es) and carrier with the necessary information.

3. Prepare a dish by aseptically dispensing 20 µL of any 510(k) cleared HEPES or MOPS buffered holding medium for use outside of incubator into the well/dish. Transfer the oocyte(s) from the culture dish to the holding buffer. Start the next step within 1 minute.

4. Make sure the contents of each vial of ES and VS are well mixed by gentle inversion several times before use.

5. Add 20 µL ES (ES1) to the drop with the oocyte(s) and leave for 3 minutes. Then add another 20 µL ES (ES2) and leave for another 3 minutes (Figure 2).

6. Add another 400 µL ES (ES3) and leave for 6-9 minutes.
The oocyte(s) will shrink and then gradually re-expand to their original size, indicating that equilibration is complete.

7. Towards the end of the equilibration time in ES, set up 2 x 250 µL of VS as shown in Figure 2.

8. Steps 9-11 should be completed within 90-110 seconds.

9. After equilibration in ES is complete, draw up some ES into the transfer pipette and transfer the specimen(s) with minimal volume from the ES into the first drop of VS (VS1) and leave for a maximum of 30 seconds.

10. Quickly, transfer the specimen(s) from VS1 to the center of the second drop of VS (VS2) and leave for a maximum of 30 seconds.

11. For the vitrification procedure, carefully transfer the oocyte(s) with minimal volume of VS from VS2 to the carrier, as recommended by the manufacturer.

If more oocytes are to be vitrified, repeat steps 3 to 11 above using fresh solutions of ES and VS.

C. Procedure using larger volumes of solution (zygotes, embryos and blastocysts)
Maximum of 6 zygotes/embryos/ blastocysts processed per dispensed media.

1. Fill the liquid nitrogen reservoir with liquid nitrogen and prepare system for storage of the vitrified specimens.

2. Label the dish(es) and carrier with the necessary information.

3. Make sure the contents of each vial of ES and VS are well mixed by gentle inversion several times before use.

4. Prepare the dish by aseptically dispensing 1mL of ES and 1 mL of VS (see Figure 3).

5. Remove the culture dish containing the specimen(s) from the incubator and check their quality.

6. Carefully transfer the specimen(s) with a minimal volume of culture medium to the ES and start the timer. Allow the specimen(s) to equilibrate for 5 to 15 minutes. The specimen(s) will shrink and then gradually re-expand to their original size, indicating that equilibration is complete.

7. Steps 8-10 should be completed within 90-110 seconds.

8. After equilibration in ES is complete, draw up some ES into the transfer pipette and transfer the specimen(s) with minimal volume from the ES to the VS.

9. Gently swirl the specimen(s) in the VS for 20-30 seconds to thoroughly mix with the VS solution. Leave for maximum 30 seconds.

10. For the vitrification procedure, carefully transfer the specimen(s) with minimal volume of VS to the carrier, as recommended by the manufacturer.

Figure 1

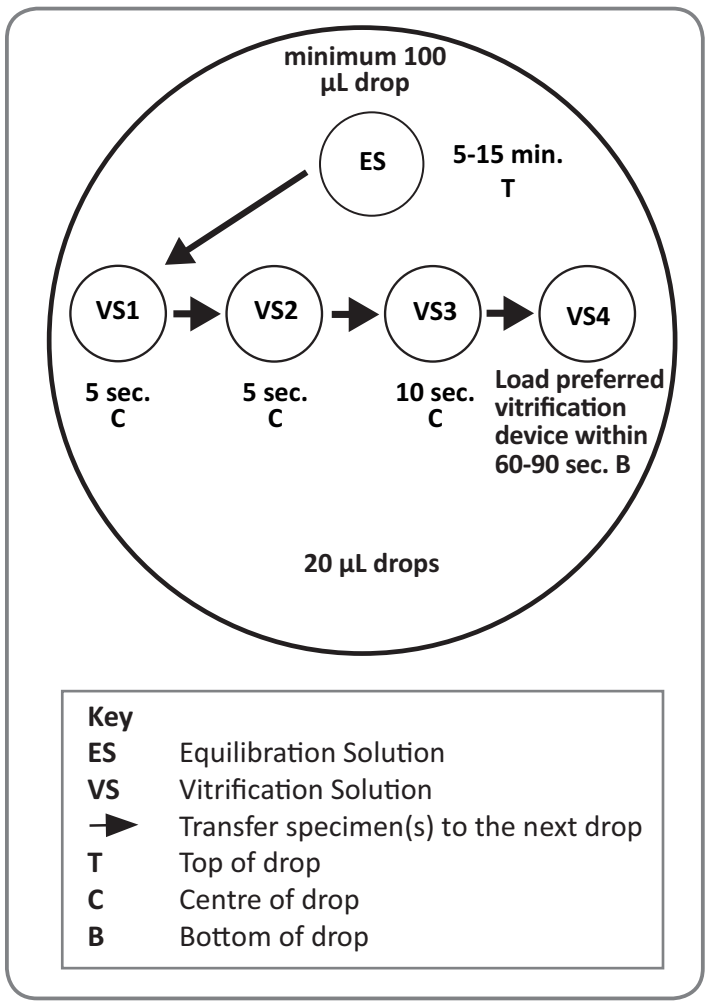

Figure 2

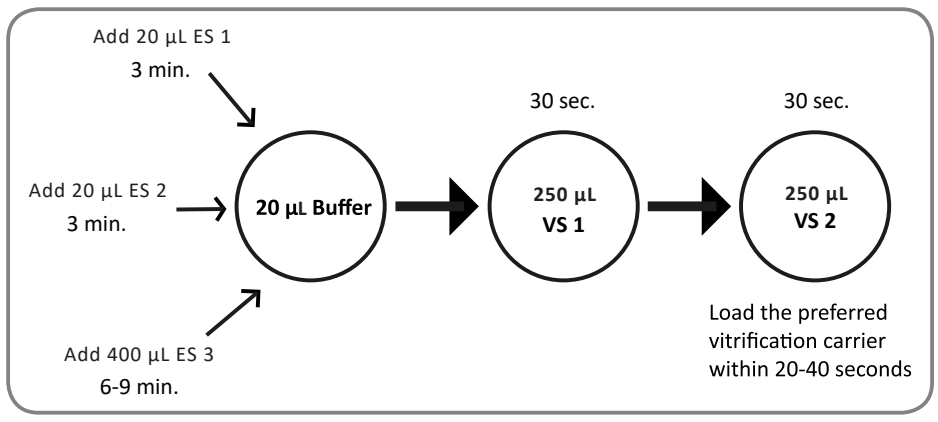

Figure 3