IFU SafeSpeed Cooling Media kit

Intended use

SafeSpeed Cooling Media is designed for ultra-rapid cooling of human oocytes and embryos by vitrification.

Products for vitrification included in the kit:

1 x Vial 1 (2ml): Washing Solution (WS)
1 x Vial 2 (2ml): Equilibration Solution (ES)
1 x Vial 3 (2ml): Vitrification Solution (VS)

Other materials needed for vitrification not included in the kit:

- Vitrification straws (SafeSpeed cryopreservation closed support, SafePreservation S.L., Spain, is recommended)
- Liquid Nitrogen container (15 cm depth)
- Metal clamps
- Sterile Petri dish, suitable for embryology use
- Aspiration system (Stripper Pipette) *
- Connector: SafePreservation provides a compatible specific connector for all aspiration systems
- Microscope
- Stopwatch or timer
- Micropipette (100-1000 µL)
- Sealer (SafeSealer, SafePreservation S.L., Spain)
- Visotub/goblet

***Note**: *Use a pipette with an internal diameter suitable for the oocytes or embryos. Capillaries of 170 µm are recommended for vitrification of oocytes and embryos, capillaries of 200 µm for early blastocysts (up to type 3 according to Gardner or ASEBIR classification) and capillaries of 290 µm for expanded blastocysts. This makes possible to optimize the volume of the solutions to achieve the best dilution condition and obtain a high survival rate*.

Chemical composition

Base components (Vial 1: Washing Solution – WS)

Phenol Red	Na-Pyr	NaOH	Sucrose
PBS	HPC	HCl	

Vial 2: Equilibration Solution (ES)
Ethylene glycol (EG) 7.5 % (v/v)
Dimethyl sulfoxide (DMSO) 7.5 % (v/v)

Vial 3: Vitrification Solution (VS)
Ethylene glycol (EG) 15 % (v/v)
Dimethyl sulfoxide (DMSO) 15 % (v/v)
Sucrose 0.5 M

Quality Control testing

Each solution of the SafeSpeed Cooling Media kit is sterilized by filtration and confirm a sterility assurance level (SAL) 10-6.

Each lot of SafeSpeed Cooling Media is tested for:
Endotoxins by LAL methodology ≤ 0.5 EU/ml
Mouse Embryo Assay (one-cell) ≥ 80 % expanded blastocyst after 96 h
Sterility test according to UNE EN ISO 11737-2
pH test: 7.2-7.6

All results are reported on a lot specific Certificate of Analysis which is available upon request.

Storage and stability instructions

The products are aseptically processed and supplied sterile.

The solutions should be stored in their original, unopened vial and refrigerated at a temperature between 2-8 °C protected from (sun) light. Do not use media that have not been store under these conditions. When stored as directed by the manufacturer the product is stable until the expiry date shown on the vial label.

The product is supplied in single-use vials.

Do not freeze before use.

Warnings and precautions

- Read the instructions for use before using the product.
- Do not re-sterilize.
- Do not re-use. This product is for single use and not to be reused due to the risk of contamination and infection.
- Not injectable.
- Do not use any vial if shows evidence of damage, leaking, suspended particles, turbidity, or has changed colour. Discard the product in accordance with the applicable standards.
- The product should not be used if the sterile package is found to be incomplete or open.
- Do not use and discard the product if it has passed its expiry date.
- Dispose of excess (not used) after warm-up.
- Each media is for same patient use.
- This product is intended to be used by professional trained personal in assisted reproductive techniques that includes the intended application for this product.
- To avoid contamination problems, aseptic handling techniques should be used.
- Additional precaution, it is recommended to carefully examine the vitrification straw when removing it from its package and check it does not show cracks or ruptures.
- Dispose the product as residual and non-reusable solutions.
- Dispose the containers and packaging as indicated to containers with solutions inside.
- It is recommended to use sealed vitrification instruments.

- Currently, clinical research literature indicates that the long-term effects of vitrification on oocytes and embryos, and on new-born infants born after cryopreservation remain unknown.
- The User facility of this device is responsible for maintaining product traceability and must comply with national regulations regarding traceability, where applicable.
- It is strongly recommended that the patient's name and the batch number of the SafeSpeed Media product being administered be recorded to ensure the patient-product relationship. Vecmedical Spain S.L. will keep the information regarding the product batch for 20 years.

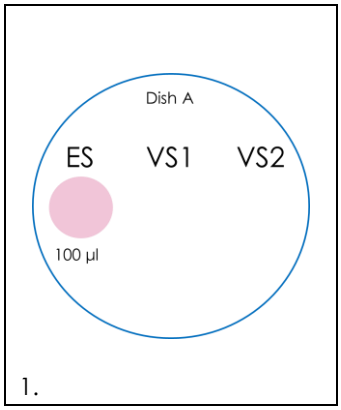
Contraindications

This product contains Ethylene glycol (EG) and dimethyl sulfoxide (DMSO), the user must take the necessary precautions to ensure that the patient is not sensitive to any of these components.

Instructions for use

Make sure that all media have their content homogenized before using them by gently inverting them twice. It is recommended to read all the steps of the vitrification procedure before starting the process.
The process should be performed at room temperature (~22-24 °C).

Illustrations



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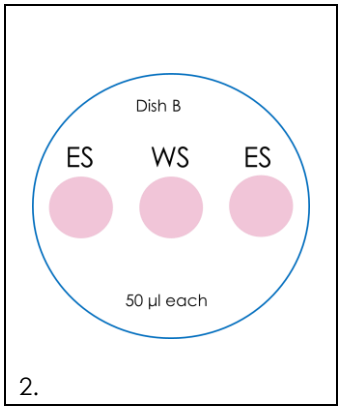

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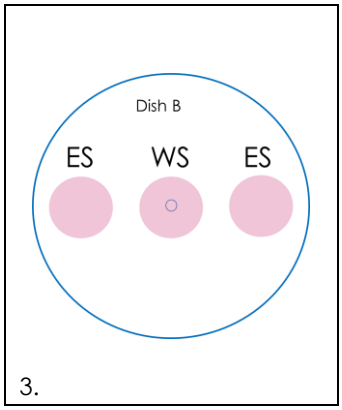

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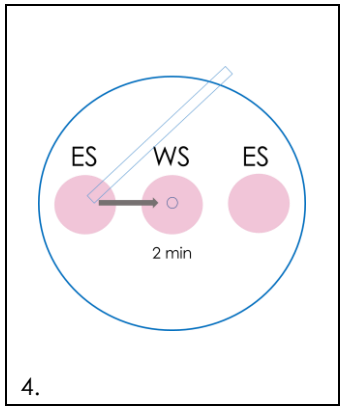

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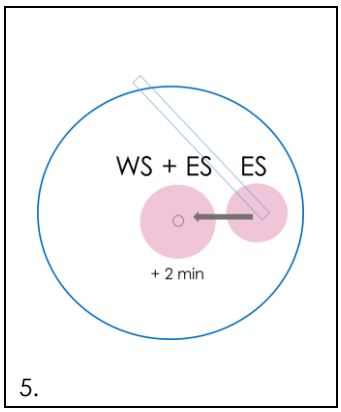

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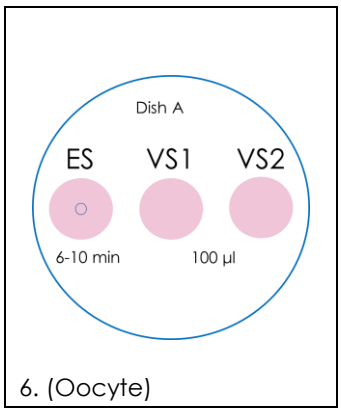
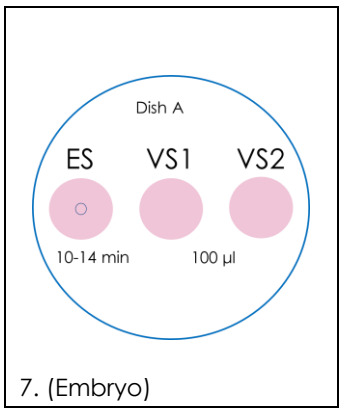
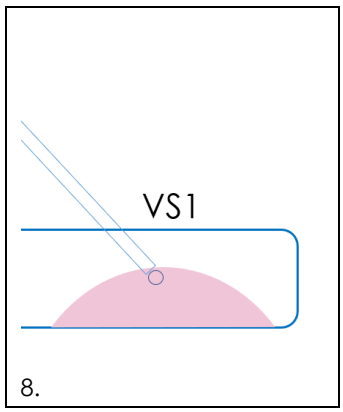


6. (Oocyte)



7. (Embryo)



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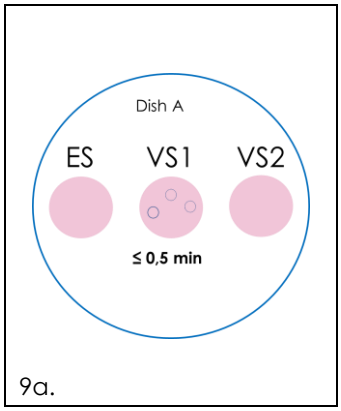
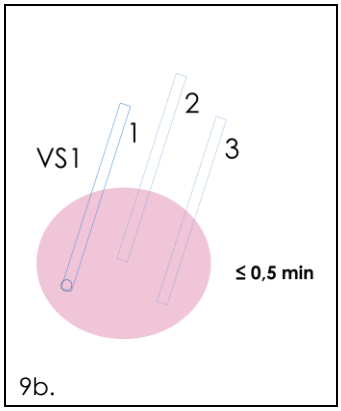


9a.



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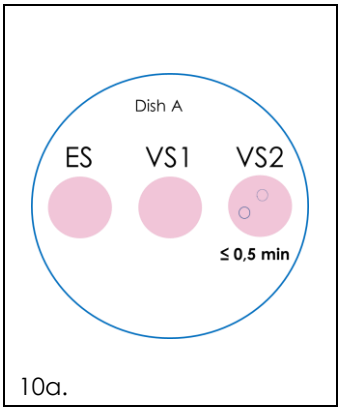

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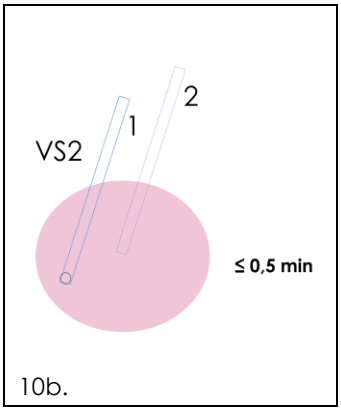

10b.

Preparation for vitrification

1. Expose vials of WS, ES and VS solutions to room temperature at least one hour before use.
2. Write the necessary information about the patient in the writing area of the vitrification straw, or stick the adhesive label containing this information.
3. Fill 90 % of the container with liquid nitrogen.
4. Take the culture plate with the oocytes/embryos out of the incubator. Check the quality of the oocytes/embryos under the microscope.

Note: *it is recommended to record the width of the perivitelline space and the thickness of the zona pellucida of the oocytes before starting the vitrification process, so as to recognise when equilibration has been reached during exposure to the ES medium*.

Equilibration

1. Label a Petri dish (dish A) with three marks: ES, VS1 and VS2.
2. Carefully invert the ES vial twice to help homogenize its contents. Form a drop of 100 µL of ES over the ES mark on the dish A (See illustration 1).
3. Set the timer. Set the timer to control steps *c*, *d* and *e* of oocyte equilibration, and/or step *a* of embryo equilibration, explained below.

<u>Oocytes equilibration</u>

Note: *for oocyte vitrification, take the cumulus cells off before beginning the process*.

a. Label another Petri dish (dish B) with three marks: WS, ES and ES. Form the following three drops on the respective marks: a drop of 50 µL of WS on the WS mark; next to this drop place a drop of 50 µL of ES on each of the two ES marks (See illustration 2).
b. Aspirate the oocyte into the tip of the stripper pipette. Place the oocyte with the minimum possible volume of culture medium over the centre of the drop of 50 µL WS from dish B (See illustration 3).
c. In the dish B, pipette the second drop of 50 µL of ES to the WS drop containing the oocyte and leave for 2 minutes (See illustration 4).
d. In the dish B, gently add the third drop of 50 µL of ES to the other two drops of ES and WS, and leave it for another 2 minutes (See illustration 5).
e. Aspirate the oocyte and place it in the drop of 100 µL of ES on the dish A. Let it rest for 6-10 minutes, until it is completely equilibrated* (See illustration 6 - oocyte).

f. In the last minutes of equilibration, carefully invert the VS vial twice to help homogenize its contents, and form a drop of 100 µL of VS over the VS1 mark and another over the VS2 mark of the dish A.

***Note**: *To complete the equilibration, it is necessary that the volume of the oocytes is completely recovered. Check that the amplitude of the perivitelline space is equal to the amplitude it had before being immersed in the ES*.

Embryos equilibration

a. Aspirate the embryo with the tip of the Stripper pipette. Place the embryo with the minimum possible volume of culture medium over the center of the 100 µL ES drop of dish A. Let it rest for 10-14 minutes (See illustration 7 - embryo).
(See "Summary of Balancing Times" below).
b. The embryo begins to contract spontaneously and then gradually returns to its original size with the infiltration of ES, indicating that the equilibration is complete.
c. In the last minutes of equilibration, carefully invert the VS vial twice to help homogenize its contents, and form a drop of 100 µL of VS over the VS1 mark and another over the VS2 mark of the dish A.

Note: *For blastocyst equilibration, wait until the perivitelline space disappears*.

Summary of equilibration time:
- *Oocyte: 6-10 minutes*
- *2PN, 4-cells or 8-cells: 10-14 minutes*
- *Morula or Blastocyst: 12-14 minutes*

***Vitrification* (for both oocytes and embryos)**

Step 1 - after completing the equilibration

1. In the dish A, aspirate the oocyte/embryo from the ES drop with the tip of the stripper pipette. Transfer the oocyte/embryo to the surface of the VS1 drop with the minimum possible volume of ES, the cells will shrink again (see illustration 8).
2. Expel only the oocyte/embryo to VS1.
3. To avoid introducing the ES residuals from the pipette into the VS1, expel the ES residuals out of the VS1 content. Aspirate fresh VS1 and expel it again out of the compartment.
4. Aspirate fresh VS1 into the pipette. Aspirate the oocyte/embryo from VS1 with the pipette tip and expel it back into the drop of VS1, repositioning it three times to remove any remaining ES. The oocyte/embryo should remain in VS1 for approximately half a minute (See illustration 9a-b).
5. Discharge the VS1 residual from the pipette out of the container (See illustration 10a).
6. Aspirate fresh VS2 into the pipette, then aspirate the oocyte/embryo of VS1 with the pipette tip.
7. Transfer the oocyte/embryo to VS2 with the minimum volume of VS1.
8. Stir around the oocyte/embryo with the pipette changing positions twice within the VS2. This operation should take about half a minute (See illustration 10b).

CAUTION: The time that oocyte/embryo is in VS (sum of VS1 plus VS2) should not exceed 1 minute.

Step 2 – loading into the vitrification device

CAUTION: Use metal clamps to avoid direct user contact with the liquid nitrogen.

9. When the oocyte/embryo is ready to be loaded into the vitrification device, carefully load the oocyte/embryo with the minimum volume of VS2 (< 0.1 µL) into the vitrification device, following the instructions for use of the vitrification device. Always perform a controlled loading of the samples under the microscope.
10. Quickly dip the vitrification straw with the samples into the liquid nitrogen container. Transfer the vitrification device to a visotube/goblet.
    CAUTION: Avoid hitting the walls and bottom of the container with the device; it could break.
11. Transfer the visotube/goblet containing the vitrification device to long-term storage tank.
    CAUTION: Do not expose the vitrification device to air until warming process.

Symbols explanation

Symbol	Description
CE 0318	(European Conformity)
	Symbol "DO NOT REUSE"
	Symbol "DO NOT RESTERILIZE"
	Symbol "DO NOT USE IF PACKAGE IS DAMAGE"
	Symbol "USE-BY DATE"
LOT	Symbol "BATCH CODE"
STERILE A	Symbol "STERILE USING ASEPTIC PROCESSING TECHNIQUES" (BY FILTRATION)
REF	Symbol "CATALOG CODE"
	Symbol "CAUTION".
	Symbol "CONSULT INSTRUCTIONS FOR USE"
	Symbol "TEMPERATURE LIMIT"
	Symbol "MANUFACTURER"

The Safety Data Sheet is available to the user.
Please contact the manufacturer for the MSDS.

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08110 Spain.
DISTRIBUTED BY: SafePreservation S.L.

REVIEW 09/2020

CE 0318