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Instructions for the Use of the global® DMSO Blastocyst Vitrification Kit

(Catalogue Numbers: DMVT-002/DMV5-005)

Caution: Federal Law (USA) restricts this device to sale by or on the order of a physician (or properly licensed practitioner).

Caution: The user should read and understand the Directions for Use, Precautions and Warnings, and be trained in the correct procedure before using the global® DMSO Blastocyst Vitrification Kit for vitrification of human blastocysts.

I. Precautions and Warnings

1. **Warning:** The long term safety of blastocyst vitrification on children born from this procedure is unknown.
2. **Warning:** The safety and effectiveness of vitrification has not been fully evaluated in human embryos that have not yet reached the blastocyst stage of development.
3. Not to be used for injection
4. This product contains human serum albumin, a derivative of human blood

   The human serum albumin used in the preparation of this product has been heated at 60°C for ten hours.

   Caution: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested for antibodies to HIV, HBc, HCV, and HTLV I/II and non-reactive for HbsAg, HCV RNA and HIV-1 RNA and syphilis. No known test methods can offer assurances that products derived from human blood will not transmit infectious agents.
5. **global® DMSO Blastocyst Vitrification Kit** solutions contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic
6. Do not use the product if:
   - the product packaging appears damaged or if the seal is broken
   - the expiry date has been exceeded
   - the product becomes discolored, cloudy, or shows evidence of particulate matter
7. To avoid problems with contamination, practice aseptic techniques.
8. Use a legally marketed carrier and storage device appropriate for blastocyst vitrification procedures.
9. 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.
10. The rate of cooling in the carrier and storage device should be between 1,800 to 20,000°C/min (Camus *et al*., 2006)
11. The global® DMSO Blastocyst Vitrification Kit is intended for single use only (the vitrification of blastocyst(s) from one patient on one day). Discard any unused product after opening.

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II. General Information

Intended Use: **global® DMSO Blastocyst Vitrification Kit** is intended for the vitrification (ultra-rapid freezing) and cryostorage of human blastocysts.

This kit is intended to be used in conjunction with the **global® DMSO Blastocyst Vitrification Warming Kit**

Composition

Base Components			
Sodium Chloride	Potassium Chloride	Calcium Chloride	Potassium Phosphate
Magnesium Sulfate	Sodium Bicarbonate	Glucose	Lactate Na Salt
Sodium Pyruvate	Glycine	L-Alanine	L-Arginine HCl
L-Asparagine	L-Aspartic Acid	L-Cystine	L-Glutamic Acid
Glycyl-Glutamine	L-Histidine	L-Isoleucine	L-Leucine
L-Lysine HCl	L-Methionine	L-Phenylalanine	L-Proline
L-Serine	L-Threonine	L-Tryptophan	L-Tyrosine
L-Valine	EDTA	Gentamicin	Phenol Red
HEPES			

Equilibration Solution	**Vitrification Solution**
Base components Dimethyl Sulfoxide (7.5% v/v) Ethylene Glycol (7.5% v/v) Human Serum Albumin (10 mg/ml)	Base components Dimethyl Sulfoxide (15% v/v) Ethylene Glycol (15% v/v) Sucrose (0.5 M) Human Serum Albumin (10 mg/ml)

Storage: Store at 2-8ºC and protected from light.

Shelf Life: Six (6) months from the date of manufacture when stored unopened at 2-8ºC and protected from light.

III. Quality Control

The solutions in the **global® DMSO Blastocyst Vitrification Kit** are membrane filtered and aseptically processed according to cGMP procedures which have been validated to meet a sterility assurance level (SAL) of 10^{-3}.

	Specification
Physiochemical tests:	
• pH – Equilibration and Vitrification Solutions	7.1-7.5
• Osmolarity – Equilibration solution (1:1 dilution) (mOsM)	1300-1750
• Osmolarity – Vitrification solution (1:3 dilution) (mOsM)	1225-1650
Biological Tests	
• LAL Endotoxin - Equilibration and Vitrification Solutions	< 1.0 EU/ml
• Sterility Test, membrane filtration	Negative
1-cell Mouse Embryo Assay: (% blastocysts at 96h of culture following step-in, step-out of vitrification and warming solutions)	≥ 80%

IV. Components of the global® DMSO Blastocyst Vitrification Kit

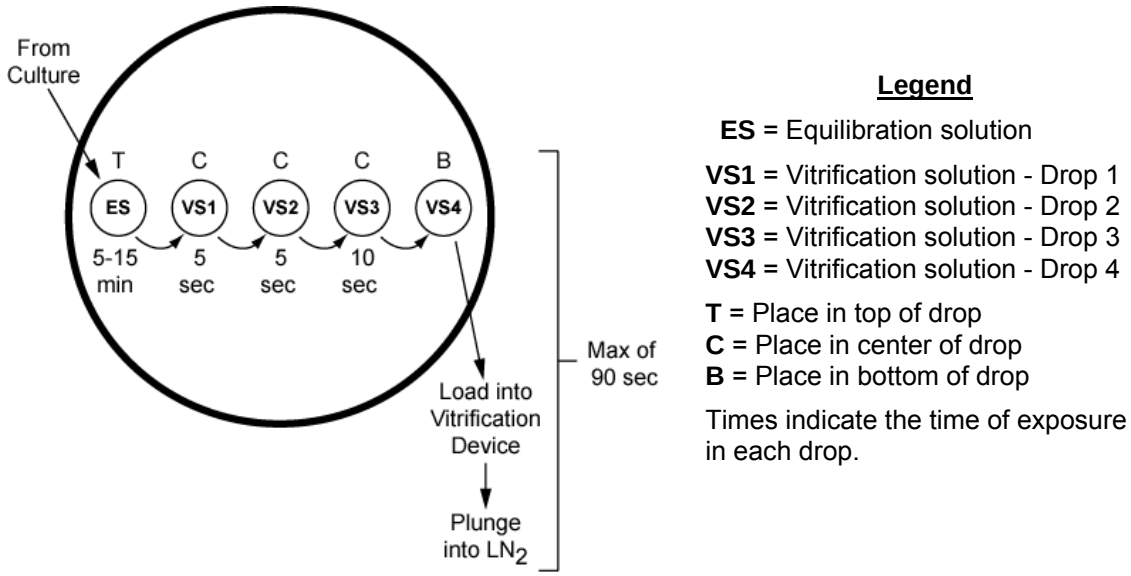
Solution	Cap Color	DMVT-002	DMV5-005
Equilibration	Blue	2 X 1.0 ml	1 X 5.0 ml
Vitrification	White	2 X 1.0 ml	1 X 5.0 ml
GPS® culture dishes		10	10

V. Required Materials Not Included in the Kit

1. Carrier devices appropriate for the vitrification of blastocysts
2. Cryotubes or goblets and cryocanes
3. Disposable gloves
4. Transfer pipettes (pulled glass pipettes or micropipette tips with an inner tip diam. of 200 um)
5. Tweezers
6. Timer or stopwatch
7. Liquid Nitrogen Reservoir (Dewar or Styrofoam container with lid, 1-2 L volume)
8. Liquid Nitrogen (sufficient volume to achieve 6 inch depth in reservoir)

VI. Directions for Use

Figure 1. Overview of the use of the **global® DMSO Blastocyst Vitrification Kit** solutions for vitrification of human blastocysts. See text for complete instructions.



1. The vitrification procedure is to be performed at room temperature (20-27°C). Bring the **Equilibration** and **Vitrification** solutions to room temperature before use. Do not use a heated microscope stage for the following procedures. Minimize exposure of specimens to light during exposure to the Equilibration and Vitrification Solutions.
2. Fill the liquid nitrogen reservoir with liquid nitrogen to a sufficient depth to submerge a cryotube or goblet on a cryocane and place near to microscope. Attach a cryotube or goblet to the bottom of the cryocane and submerge in the liquid nitrogen in preparation for storage of the vitrified specimens.
3. Determine the number of embryos to be vitrified. Whenever possible, use only the best quality expanded blastocyst(s) for vitrification.

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4. Label each culture dish and carrier/storage device to be used with necessary information.
5. Make sure the contents of each vial of **Equilibration** and **Vitrification** are well mixed by gentle inversion several times before use.
6. Aseptically pipette 20 µl of **Equilibration** solution **(ES)** into the inverted lid of a Petri dish as shown in Figure 1.
7. Remove the culture dish with the blastocyst(s) from the incubator and check the quality of the embryos.
8. Carefully transfer the blastocyst(s) (no more than two in one procedure) with a minimal volume of culture medium (or holding medium) to the top of the drop of **Equilibration** solution (**ES**) and start the timer. Allow the blastocyst to equilibrate in **ES** during freefall for 5 to 15 minutes. The blastocyst will shrink and then gradually re-expand to its original size, indicating that equilibration is complete.
9. While the blastocyst(s) are equilibrating in **ES**, dispense four 20 µl drops of the **Vitrification** solution into the inverted Petri dish lid (**VS1**, **VS2**, **VS3**, **VS4**), as shown in Figure 1.

IMPORTANT: The following steps (10-15) must be completed in 90-110 seconds.

10. After equilibration in ES is complete, draw up some of the **Vitrification** solution **(VS1)** into the transfer pipette and transfer the blastocyst(s) with minimal volume from the ES into the center of the first drop of **Vitrification** solution (**VS1**), and hold for 5 seconds.
11. Quickly transfer the blastocyst(s) from VS1 to the center of the second drop of **Vitrification** solution (**VS2**), and hold for 5 seconds.
12. Quickly transfer the blastocyst(s) from VS2 to the center of the third drop of **Vitrification** solution (**VS3**), and hold for 10 seconds.
13. Finally, transfer the blastocyst(s) from the third drop of VS3 to the bottom of the fourth drop of **Vitrification** solution (**VS4**).
14. For the vitrification procedure, follow the instructions that accompany the vitrification carrier device. Carefully transfer the blastocyst(s) from VS4 into the vitrification device to be used and proceed as instructed using the Directions for Use that accompany the carrier device.
15. **Reminder**: The amount of time between first placing the blastocyst(s) in the first drop of **Vitrification** solution (**VS1**) and immersion into liquid nitrogen should not exceed the time you have determined to be optimal for this procedure and certainly should not be more than 110 seconds.
16. **Storage in liquid nitrogen:** (all these procedures should be done with the vitrified sample fully immersed under liquid nitrogen to prevent any inadvertent warming). Transfer the vitrified sample into an appropriately labeled cryotube or goblet attached to a cryocane with another goblet inverted over the top to act as a cap and transfer the cryocane to a storage tank of liquid nitrogen.
17. If more blastocysts are to be vitrified, repeat steps 4 through 16 above, using fresh drops of **Equilibration Solution** and **Vitrification Solution**, in a new dish lid

VII. References

- Camus A, Clairaz P, Ersham A, Van Kappel AL, Savic G, Staub C (2006) [The comparison of the process of five different vitrification devices]. *Gynecol Obstet Fertil* **34**, 737-45.
- Kuwayama M, Vajta G, Ieda S, Kato O (2005) Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. *Reprod Biomed Online* **11**, 608-14.