LifeGlobal® GROUP

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

(Catalogue Numbers: DMWR-003/DMW5-005)

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 Warming 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 Warming 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 **global® DMSO Blastocyst Vitrification Warming Kit** is intended for single use only (the rehydration of vitrified 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 Warming Kit** is intended for the recovery and rehydration of human blastocysts that have been vitrified using the **global® DMSO Blastocyst Vitrification 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			

Warm 1	**Warm 2**	**Warm 3**
Base components Sucrose (1.0 M) HSA* (10 mg/ml)	Base components Sucrose (0.5 M) HSA (10 mg/ml)	Base components HSA (10 mg/ml)

*Human Serum Albumin

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 - Warm 1, Warm 2 & Warm 3	7.1-7.5
• Osmolarity – Warm 1 (1:1 dilution) (mOsM)	590-660
• Osmolarity – Warm 2 (mOsM)	800-875
• Osmolarity – Warm 3 (mOsM)	270-300
Biological Tests	
• LAL Endotoxin - Warm 1, Warm 2 & Warm 3 (EU/ml)	< 1.0
• Sterility Test, membrane filtration	Negative
1-cell Mouse Embryo Assay: (% blastocysts at 96h of culture following sequential step-in and step-out of vitrification and warming solutions)	≥ 80%

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IV. Components of the global® DMSO Blastocyst Vitrification Warming Kit

Solution	Cap Color	DMWR-003	DMW5-005
Warm 1	Green	1 X 1.0 ml	1 X 5.0 ml
Warm 2	Yellow	1 X 1.0 ml	1 X 5.0 ml
Warm 3	Red	2 X 1.0 ml	2 X 5.0 ml
GPS® culture dishes		10	10

V. Required Materials Not Included in the Kit

1. Disposable gloves
2. Transfer pipettes (pulled glass pipettes or micropipette tips with an inner tip diameter of 200 um)
3. Tweezers
4. Scissors or scalpel
5. Timer or stopwatch
6. Liquid Nitrogen Reservoir - Dewar or Styrofoam container with lid, 1-2 liter volume
7. Liquid Nitrogen – a sufficient volume to cover the cryotube or goblet containing the vitrified blastocyst(s).
8. Culture medium: e.g. **global®**, supplemented with protein, prepared, and pre-equilibrated in a culture dish prior to thawing the blastocysts.

VI. Directions for Use

Preparation

1. The rehydration procedures are to be performed at 20-27°C.
2. Minimize exposure of specimens to light during incubation in the warming solutions.
3. Warm the **global® DMSO Blastocyst Vitrification Warming Kit** solutions **(Warm 1**, **Warm 2** and **Warm 3**) to 20-27°C before use.
4. Refer to the manufacturer's instruction for use of the carrier device that has been used for specific instructions pertaining to that device
5. Fill the liquid nitrogen reservoir with liquid nitrogen to a sufficient depth to completely submerge a cryotube or goblet containing the carrier device used and place near to the liquid nitrogen tank containing the vitrified samples to be warmed.
6. Remove the cryocanes or goblets containing the carrier device with vitrified blastocyst(s) and quickly transfer them to the reservoir containing liquid nitrogen, keeping the carrier device under liquid nitrogen at all times.
7. Place the liquid nitrogen reservoir close to the microscope for rapid manipulation.
8. Label a Petri dish lid, with all necessary information.
9. Make sure the contents of each vial of **Warm 1**, **Warm 2**, and **Warm 3** are well mixed by gentle inversion several times before use.
10. Aseptically dispense a 20 µl drop of **Warm 1** into the inverted dish lid (**W1**a), as shown in Figure 1.
11. Dispense two 20 µl drops of **Warm 2** into the inverted dish lid (**W2a** & **W2b**), as shown in Figure 1.

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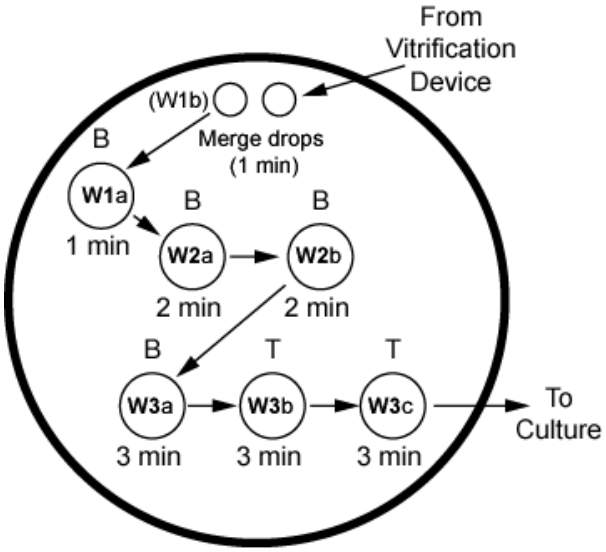
Figure 1. Overview of the use of the **global® DMSO Blastocyst Vitrification Warming Kit** solutions in a GPS™ dish for the rehydration of human blastocysts that have been vitrified using the **global® DMSO Blastocyst Vitrification Kit**. See text for complete instructions.



Legend

W1a = Warm 1 – Drop a
W1b = Warm 1 – Drop b

W2a = Warm 2 - Drop a
W2b = Warm 2 - Drop b

W3a = Warm 3 - Drop a
W3b = Warm 3 - Drop b
W3c = Warm 3 - Drop c

B = Bottom of drop
T = Top of drop

Times indicate the time of exposure in each drop.

Thawing and Rehydration

12. Using tweezers, locate the carrier device on the cane in the liquid nitrogen reservoir. Only one carrier device at a time is to be processed.
13. Carefully remove the carrier device from the cane, keeping the part containing the blastocyst(s) under the surface of the liquid nitrogen.
14. Follow the directions for use that accompany the carrier device used to warm and open the vitrification device, and then immediately dispense the contents of the device (approximately 1 µl) onto the dish lid, as shown in Figure 1. Avoid forming bubbles when dispensing the contents of the device.
15. Draw up approximately 1 µl of solution from **W1a**, and dispense it adjacent to the drop containing the blastocyst(s), as shown in Figure 1 (**W1b**).
16. Use the vitrification device to merge the two drops, and then allow gradual mixing for 1 minute.
17. Draw up a small volume from **W1a** into the transfer pipette and then use it to pick up and transfer the blastocyst(s), in a minimal volume, from **W1b** to the bottom of **W1a**.
18. Hold the blastocyst(s) in **W1a** for 1 minute. The blastocyst(s) will shrink and float to the top of the drop.
19. **Note:** After each transfer of the embryos described in the following steps, eject any remaining fluid in the transfer pipette and draw up some solution from the next drop, prior to the next manipulation. Avoid creating bubbles during transfers.
20. Transfer the blastocyst(s) from **W1a** to the bottom of **W2a.**
21. Hold the blastocyst(s) in **W2a** for 2 minutes.
22. Transfer the blastocyst(s) from **W2a** to the bottom of **W2b**
23. Hold the blastocyst(s) in **W2b** for 2 minutes.
24. **Note:** The blastocyst(s) will remain shrunken in **W2a** and **W2b**.

25. During the holding time in **W2b**, dispense three 20 µl drops of **Warm 3** into the inverted dish lid as shown in Figure 1 (**W3a**, **W3b** & **W3c**).
26. Transfer the blastocyst(s) from **W2b** to the bottom of **W3a**
27. Hold the blastocyst(s) in **W3a** for 3 minutes.
28. Transfer the blastocyst(s) from **W3a** to the top of **W3b**
29. Hold the blastocyst(s) in **W3b** for 3 minutes.
30. Transfer the blastocyst(s) from **W3b** to the top of **W3c**
31. Hold the blastocyst(s) in **W3c** for 3 minutes.
32. Finally, transfer the blastocyst(s) to a dish of pre-equilibrated appropriate culture medium and incubate in a CO_2 incubator at 37°C for 3-4 hours to allow for further recovery prior to further manipulations and/or transfer. .
33. If more blastocysts are to be warmed, repeat steps 8 through 32 above, using fresh drops of **Warm 1**, **Warm 2**, and **Warm 3**, 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.