



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

(Catalogue Numbers: DMV5-005)

### PRECAUTIONS AND WARNINGS

1. **Caution:** Federal Law (USA) restricts this device to sale by or on the order of a physician (or properly licensed practitioner).
2. **Caution:** The user should read and understand the Instructions for Use, Precautions and Warnings, and be trained in the correct procedure before using global® DMSO Blastocyst Vitrification Kit for vitrification of human blastocysts.
3. **Warning:** The long term safety of blastocyst vitrification on children born from this procedure is unknown.
4. **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.
5. Not to be used for injection.
6. Do not resterilize.
7. 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
8. 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:** Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation/removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens. There are no reports of virus transmissions with albumin manufactured to European Pharmacopoeia specifications by established processes. It is strongly recommended that every time that global® DMSO Blastocyst Vitrification Kit is administered to a patient, the name and batch number of the product are recorded in order to maintain a link between the patient and the batch of the product.
9. global® DMSO Blastocyst Vitrification Kit contains the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.
10. To avoid problems with contamination, practice aseptic techniques.
11. Use a legally marketed carrier and storage device appropriate for blastocyst vitrification procedures.
12. 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.
13. The rate of cooling in the carrier and storage device should be between 1,800 to 20,000°C/min. (Camus *et al.*, 2006)
14. The global® DMSO Blastocyst Vitrification Kit is intended **for single use only** (the vitrification of blastocysts) from one patient on one day). Discard any unused product after opening.

### GENERAL INFORMATION

#### Indications for Use

For vitrification (ultra-rapid freezing) and cryostorage of human blastocysts.

#### Storage and Shelf Life

Store at 2-8°C and protected from light. One (1) year from the date of manufacture.



## Disposal Consideration

Treat or dispose of waste material in accordance with all local state/provincial, and national requirements. Dispose with laboratory waste.

## 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
Phenol Red	HEPES	Gentamicin Sulfate* (10 µg/ml)		

### Equilibration Solution

Dimethyl Sulfoxide (7.5% v/v)  
Ethylene Glycol (7.5% v/v)  
Human Serum Albumin\* (10 mg/ml)

### Vitrification Solution

Dimethyl Sulfoxide (15% v/v)  
Ethylene Glycol (15% v/v)  
Sucrose (0.5 M)  
Human Serum Albumin\* (10 mg/ml)

\*from therapeutic-grade source material

## QUALITY CONTROL SPECIFICATIONS

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  $10^{-3}$ .

Assay (performed for each batch)	Specification
<b>MEDIA</b>	
<b>Physicochemical Tests</b>	
pH – Equilibration and Vitrification Solutions	7.1-7.5
Osmolality – Equilibration Solution	2600-3500 mOsm
Osmolality – Vitrification Solution	4900-6600 mOsm
<b>Biological Tests</b>	
Endotoxin (LAL) – Equilibration and Vitrification Solutions	≤ 1.0 EU/ml
Sterility Test (bacterial and fungal screen, SAL $10^{-3}$ )	PASS
<b>Biological Assays</b>	
1-cell Mouse Embryo Assay (% expanded blastocysts at 96 h of culture following step-in, step-out of vitrification and warming solutions)	≥ 80%
<b>EMBRYO GPS® DISHES</b>	
<b>Physicochemical Tests</b>	
Endotoxin (LAL)	< 20 EU/device
<b>Biological Tests</b>	
1-cell Mouse Embryo Assay (% expanded blastocysts after 96 h)	≥ 80%

## INSTRUCTIONS FOR USE

- The vitrification procedure and all manipulation of blastocysts are carried out at room temperature (20-27°C). Bring the Equilibration and Vitrification solutions to room temperature and mix gently each vial before use.
- Fill the liquid nitrogen reservoir with liquid nitrogen to a sufficient depth to submerge a goblet on a cryocane and place near to microscope.
- Follow the instructions that accompany the vitrification carrier device. Label with necessary information.
- Aseptically pipette 20 µl of Equilibration Solution (ES) into a Petri dish.



5. Transfer the blastocyst(s) (no more than 2) with a minimum amount of culture media the drop of ES. Allow the blastocyst(s) to equilibrate for 5 to 15 minutes.
6. While the blastocyst(s) are equilibrating in ES, dispense four 20 µl drops of the Vitrification Solution (VS) into the Petri dish (VS1, VS2, VS3 and VS4). Important: The amount of time between first placing the blastocyst(s) in VS1 and immersion into liquid nitrogen should not exceed the time 110 seconds.
7. Aspirate some of VS1 in transfer pipette and transfer the blastocyst(s) into the drop VS1 and hold for 5 seconds.
8. Quickly transfer the blastocyst(s) from VS1 to VS2, and hold for 5 seconds.
9. Quickly transfer the blastocyst(s) from VS2 to VS3, and hold for 10 seconds.
10. Finally, transfer the blastocyst(s) from VS3 to VS4. Load blastocyst(s) from VS4 into the vitrification device.
11. For the vitrification procedure, follow the instructions that accompany the vitrification carrier device.

**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 goblet attached to a cryocane. Transfer the cryocane to a storage tank of liquid nitrogen.














If more blastocysts are to be vitrified, repeat step 4-11 using fresh drops of ES and VS in a new dish.

## 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.

## SYMBOLS

	<b>RX Only</b>				
Sterile Using Aseptic Processing Techniques	By Prescription Only	Catalogue Number	Batch Code	Consult Instructions For Use	Manufacturer
					
Keep Away From Sunlight	Temperature Limitation	Authorized Representative in the European Community	Use By	Do Not Reuse	GS1 DataMatrix Barcode
					
Do Not Resterilize	European Conformance (notified body)				