



Instructions for the Use of global[®] Blastocyst Fast Freeze[®] Kit

(Catalogue Numbers: GFV5-005)

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[®] Blastocyst Fast Freeze[®] Kit for cryopreservation of human blastocysts.
3. **Warning:** The long term safety of blastocyst cryopreservation on children born from this procedure is unknown.
4. **Warning:** This kit **is not intended to be used** for the cryopreservation of human oocytes, or for 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[®] Blastocyst Fast Freeze[®] 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[®] Blastocyst Fast Freeze[®] 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 freezing straws appropriate for blastocyst cryopreservation procedures and follow the manufacturer's instructions for labeling, handling and heat-sealing the straws.
12. The global[®] Blastocyst Fast Freeze[®] Kit is intended for **single use only** (the cryopreservation of the blastocysts from one patient on one day). Discard any unused product after opening.

GENERAL INFORMATION

Indications for Use

For cryopreservation of 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	Human Serum Albumin* (20 mg/ml)		Gentamicin Sulfate* (10 µg/ml)

*from therapeutic-grade source material

global® Blastocyst Fast Freeze® Solutions 1, 2, and 3 contain increasing concentrations of glycerol and ethylene glycol together with the base components.

QUALITY CONTROL SPECIFICATIONS

Assay (performed for each batch)	Specification
MEDIA	
Physicochemical Tests	
pH – Fast Freeze® Solution 1	7.1-7.5
pH – Fast Freeze® Solution 2	7.1-7.5
pH – Fast Freeze® Solution 3	7.1-7.5
Osmolality – Fast Freeze® Solution 1	1400-2000 mOsM
Osmolality – Fast Freeze® Solution 2	4900-6600 mOsM
Osmolality – Fast Freeze® Solution 3	7800-10600 mOsM
Biological Tests	
Endotoxin (LAL) – Fast Freeze® Solution 1	≤ 1.0 EU/ml
Endotoxin (LAL) – Fast Freeze® Solution 2	≤ 1.0 EU/ml
Endotoxin (LAL) – Fast Freeze® Solution 3	≤ 1.0 EU/ml
Sterility Test (bacterial and fungal screen, SAL 10 ⁻³)	PASS
Biological Assays	
Mouse Embryo Assay (% re-expanded blastocysts at 24 h of culture)	≥ 80%
UNIVERSAL GPS® DISHES	
Physicochemical Tests	
Endotoxin (LAL)	< 20 EU/device
Biological Tests	
1-cell Mouse Embryo Assay (% expanded blastocysts after 96 h)	≥ 80%

INSTRUCTIONS FOR USE

Note: Only full or expanded blastocysts of good quality, with a visible/well organized cavity and a distinct ICM or at the hatching/hatched stage should be chosen for cryopreservation.

- The cryopreservation procedure is to be performed at room temperature (20-25°C). Bring the vials of global® Blastocyst Fast Freeze® Solutions 1, 2 and 3 (F1, F2 and F3) to room temperature before use. Mix gently each vial before use.

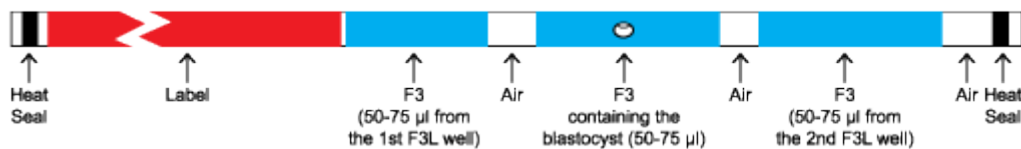


2. Label a goblet or cryotube with the information of the patient.
3. Fill a liquid nitrogen reservoir with liquid nitrogen and keep container close to working area. Always maintain sufficient liquid nitrogen in the reservoir.
4. Prepare a label for each straw according to standard laboratory protocols. It is advisable to load only one blastocyst per straw.
5. Place the label at the end of the straw over the cotton plug. Use a stylette to push the cotton plug down past the label until approximately half of the cotton plug is visible in the straw.
6. For each blastocyst, label 3 of the smaller outer wells of the Universal GPS® dish as F1, F2 and F3. Label the two larger inner wells as F3R (to be used to rinse the freezing straw) and two of the smaller outer wells as F3L (to be used to load the freezing straw).
7. Pipette a 100 µl drop of F1 into the well marked F1 in the Universal GPS® dish.
8. Transfer one blastocyst with minimal volume of culture media from the culture dish, into the drop of F1 on the labeled Universal GPS® dish. Use the transfer pipette to gently move the blastocyst across the drop, away from the unloading point, in order to dilute any carryover of culture medium.
9. Hold the blastocyst in F1 for 5 minutes.
10. Before the 5 minutes are finished, pipette a 100 µl drop of F2 into well marked F2 in the dish.
11. Load pipette with F2 and transfer the blastocyst from the drop F1 to the F2 drop. Hold the blastocyst in F2 for 5 minutes.
12. Meanwhile:
 - a. Pipette 100 µl of F3 into the well marked F3, and the two wells marked F3L, and 150 µl of F3 into each of the wells marked F3R.
 - b. Prepare the freezing straw by connecting a 1 ml syringe or other straw-loading device to the label end of the straw.
 - c. Rinse the straw with 100 µl of F3 taken from on the F3R wells of the dish. Expel media. If desired, the straw can be rinsed again, using F3 from the other F3R well.
 - d. Load the straw with 50-75 µl of F3 from the first F3L well, followed by 0.5 cm air, and then another 20-25 µl of F3. Set the syringe and straw aside, keeping it in a horizontal position.
13. Transfer the blastocyst from F2, into the F3 well. The total time from transfer of blastocyst to F3 to complete plunge of straw is 60-90 seconds.

Loading the Blastocyst into the Straw and Heat sealing

- a. Using the pre-loaded syringe, pick up the blastocyst in approximately 50 µl of F3 and aspirate air to produce the second air column (approximately 0.5 cm). Then aspirate 50-75 µl of F3 from the second F3L well.
- b. Remove the syringe, keeping the straw horizontal.
- c. Seal both ends of the straw with an appropriate heat sealer, following the manufacturer's instructions (proper sealing is crucial for a successful cryopreservation, thus optimize the temperature of the sealer for obtaining adequate sealing; do not heat seal on the cotton plug). **Figure 1**

Figure 1. Schematic representation of the method of loading the freezing straw for blastocyst cryopreservation using the global® Blastocyst Fast Freeze® Kit.



Note: Not to scale. See text for complete instructions.

Cooling and Storage of the Straw














14. Between 60 and 90 seconds after placing the blastocyst in the F3 well, plunge the straw into liquid nitrogen (−196°C) and place it into the cooled goblets, which is submerged in liquid nitrogen and has been pre-labeled with the information of the patient.
15. Repeat steps 1-14 for remaining blastocysts.
16. Transfer the cryocane to the freezing tank for long-term storage.

References

- Stachecki JJ, Cohen J (2008) S3Vitrification System: A novel approach to blastocyst freezing. *J. Clin. Embryol.* **11**, 5-14.
- Stachecki JJ, Garrisi J, Sabino S, Caetano JP, Wiemer KE, Cohen J (2008) A new safe, simple and successful vitrification method for bovine and human blastocysts. *Reprod Biomed Online* **17**, 360-7.



SYMBOLS

	RX Only				
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)				