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CE 0086 Instructions for the Use of global® Blastocyst Fast Freeze® Thawing Kit

(Catalogue Numbers: GFT5-055)

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® Thawing Kit for the thawing and recovery of human blastocysts that have been cryopreserved using global® Blastocyst Fast Freeze® Kit.
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 rehydration 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® Thawing 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® Thawing 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. The global® Blastocyst Fast Freeze® Thawing Kit is intended for **single use only** (the rehydration of the blastocysts from one patient on one day). For use with human blastocysts that have been cryopreserved using the global® Blastocyst Fast Freeze® Kit. Discard any unused product after opening.

GENERAL INFORMATION

Indications for Use

Rehydration of blastocysts that have been cryopreserved using the global® Blastocyst Fast Freeze® Kit.

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* (10 mg/ml)		Gentamicin Sulfate* (10 µg/ml)

*from therapeutic-grade source material

global® Blastocyst Fast Freeze® Thawing Solutions 1, 2, 3, 4 and 5 contain decreasing concentrations of sucrose together with the base components.

QUALITY CONTROL SPECIFICATIONS

Assay (performed for each batch)	Specification
MEDIA	
Physicochemical Tests	
pH – Thawing Solution 1	7.1-7.5
pH – Thawing Solution 2	7.1-7.5
pH – Thawing Solution 3	7.1-7.5
pH – Thawing Solution 4	7.1-7.5
pH – Thawing Solution 5	7.1-7.5
Osmolality – Thawing Solution 1	1100-1325 mOsM
Osmolality – Thawing Solution 2	650-755 mOsM
Osmolality – Thawing Solution 3	400-525 mOsM
Osmolality – Thawing Solution 4	365-405 mOsM
Osmolality – Thawing Solution 5	270-300 mOsM
Biological Tests	
Endotoxin (LAL) – Thawing Solution 1	≤ 1.0 EU/ml
Endotoxin (LAL) – Thawing Solution 2	≤ 1.0 EU/ml
Endotoxin (LAL) – Thawing Solution 3	≤ 1.0 EU/ml
Endotoxin (LAL) – Thawing Solution 4	≤ 1.0 EU/ml
Endotoxin (LAL) – Thawing Solution 5	≤ 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%

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INSTRUCTIONS FOR USE

1. Twenty-four hours prior to thawing of the blastocysts, prepare 2 labeled culture dishes per patient (1 rinsing dish and 1 culture dish). The dishes should contain microdroplets of approximately 20-50 µl of the appropriate blastocyst culture medium overlaid with mineral oil. The dishes should be equilibrated overnight in a CO_2 incubator at 37°C.

2. The thawing procedure is to be performed at room temperature (20-25°C). Bring the mineral oil and the vials of global® Blastocyst Fast Freeze® Thawing Solutions 1, 2, 3, 4 and 5 (T1, T2, T3, T4 and T5) to room temperature before use. Mix gently all the vials.

3. Fill the liquid nitrogen reservoir with liquid nitrogen. Transfer the goblets or cryotubes with the blastocysts to be thawed from the liquid nitrogen storage container to the liquid nitrogen reservoir and place it close to the microscope and the 30°C water-bath.

4. For each blastocyst to be thawed, label 5 of the smaller outer wells of one (1) Universal GPS® dish as T1, T2, T3, T4 and T5. Label the lid of the dish as T1R. Label the two larger inner wells of the dish as T1X; these will be used to provide extra T1 to recover the blastocyst from the freezing straw, if necessary.

5. Pipette 400 µl of T1 into the dish lid (T1R) and 150 µl of T1 into each of the two larger inner wells of the dish (T1X).

6. Pipette 100 µl drops of each of T1, T2, T3, T4 and T5 into the appropriate wells of the dish and overlay them with mineral oil.

7. Remove the straw containing the blastocyst from liquid nitrogen and allow it to thaw in air, for 5 seconds and then in a 30°C water-bath for 10 seconds (cotton plug up). Wipe the condensation off the straw.

8. Place the dish lid under the microscope stage and, using scissors, cut the straw above the heat seal. Then cut the opposite end just below the ID label and through the center of the cotton plug.

9. Focus the microscope on the end of the straw and use a stylette to expel the contents of the straw into the 400 µl drop of T1 in the lid (T1R).

10. Locate the blastocyst in the T1R drop and set a timer for 3 minutes.

    Note: if the blastocyst cannot be located in the T1R drop, rinse the straw with T1 from T1X wells of the dish and repeat until the blastocyst is found.

11. Gently agitate the dish lid on the surface of the laminar flow hood for 30 seconds, in order to dilute the cryoprotectants and allow the blastocyst to settle at the bottom of the drop.

12. Transfer the blastocyst to T1 and hold the blastocyst in the T1 well until the 3 minutes are finished.

13. Load transfer pipette with solution T2 and transfer blastocyst with a minimum volume of T1 to T2 well. Hold blastocyst in T2 for 5 minutes.

14. Load transfer pipette with solution T3 and transfer blastocyst with a minimum volume of T2 to T3 well. Hold blastocyst in T3 for 5 minutes.

15. Load transfer pipette with solution T4 and transfer blastocyst with a minimum volume of T3 to T4 well. Hold blastocyst in T4 for 5 minutes.

16. Load transfer pipette with solution T5 and transfer blastocyst with a minimum volume of T4 to T5 well. Move the rehydration dish to a warm surface (at 37°C) and hold the blastocyst in T5 for 5 minutes.

17. Rinse the blastocyst in culture media several times before placing them in the pre-equilibrated culture dish. Return the dish to a CO_2 incubator at 37°C.

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.

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SYMBOLS

STERILE A	**RX Only**	REF	LOT		
Sterile Using Aseptic Processing Techniques	By Prescription Only	Catalogue Number	Batch Code	Consult Instructions For Use	Manufacturer
	8°C 2°C	EC REP			
Keep Away From Sunlight	Temperature Limitation	Authorized Representative in the European Community	Use By	Do Not Reuse	GS1 DataMatrix Barcode
STERILIZE	CE 0086				
Do Not Resterilize	European Conformance (notified body)				