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Instructions for the Use of global® total™

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 **global® total™** for human embryo culture or transfer.

I. Precautions and Warnings

1. Not to be used for injection
2. 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.
3. **global® total™** contains the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.
4. 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
5. To avoid problems with contamination, practice aseptic techniques.
6. Discard unused medium within 7 days of opening.

II. General Information

Indications for Use: Culture of human embryos from zygote to blastocyst, embryo transfer

Catalogue Nos: LGGT-030 (30 ml), LGGT-060 (60 ml), LGGT-100 (100 ml)

Principle: A bicarbonate-buffered protein-supplemented medium replete with glucose, lactate, pyruvate and all 20 amino acids is optimal to support the growth and development of human embryos *in vitro*.

Composition

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

Human Serum Albumin* (4.4 mg/ml)
Human α- and β-globulins* (0.6 mg/ml)
*from therapeutic-grade source material

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

Shelf Life: No more than 10 weeks from the date of manufacture when stored unopened at 2-8ºC and protected from light. For best results, use within four weeks.

Quality Control Specifications

	Specification
Physiochemical tests:	
• pH (with 5% CO_2)	7.2-7.4
• Osmolality	260-270 mOsM
Biological Tests	
• LAL Endotoxin	<0.5 EU/ml
• Sterility Test, membrane filtration	Negative
1-cell Mouse Embryo Assay (% expanded blastocysts at 96h of culture)	≥80%

III. Storage

After each time the original bottle is opened recap the bottle tightly and store at 2-8ºC, protected from light

IV. Special Note on the CO_2 Concentration in the Incubator

In most cases, a 5-7% concentration of CO_2 in the incubator will produce a pH of 7.2 to 7.4 in **global® total™**. However, the exact concentration of CO_2 required to produce the optimum pH of approximately 7.30 (7.27-7.33) depends on several factors, including the physical characteristics of incubator and the altitude. Consequently, we strongly recommend that each laboratory determine and use the concentration of CO_2 that is required to produce a pH of 7.30 in **global® total™**.

V. Instructions for Use

1. Prepare culture dishes containing 25-100 µl droplets or in larger volumes (0.5-1.0 ml) of **global® total™** under oil, according to general laboratory practice.
2. Before introducing the embryos, place the culture dishes in the incubator for sufficient time to ensure CO_2 and temperature equilibration. Depending on the exact configuration, this may take from 24-48 hours. Equilibration will require less time if the oil and medium have been pre-equilibrated.
3. On Day 1, place the zygotes into the equilibrated **global® total™**. Culture the embryos for 48 h (Day 3, 4-8 cell stage).
4. For further culture to the blastocyst stage, transfer the cleavage-stage embryos to fresh droplets or larger volumes of **global® total™**, and culture to Day 5. For further culture to Day 6, transfer the embryos to fresh droplets or larger volumes of **global® total™**.
5. For transfer on Day 3 (cleavage stage) or Day 5/6 (blastocyst stage) wash the embryos, according to general laboratory practice, and transfer to the uterus in 20-30 µl of equilibrated **global® total™**.
6. Immediately prior to transfer, rinse the transfer catheter with **global® total™**.

VI. Medium Renewal

As noted above, In general, we recommend that the embryos should be moved to fresh dishes of **global® total™** every 48 hours. However it may be possible to maintain embryos in the same droplets or larger volumes of medium for 4 days or longer, depending on the air quality and other environmental conditions in the laboratory and in the incubator (See Reed *et al.*, 2009; 2010).

VII. References

Reed ML, Hamic A, Thompson DJ, Caperton CL (2009) Continuous uninterrupted single medium culture without medium renewal versus sequential media culture: a sibling embryo study. *Fertil Steril* **92**, 1783-6.

Reed ML, Hamic A, Thompson DJ, Caperton CL (2010) Challenging traditional embryo culture techniques with a simplified, continuous single medium protocol. *J. Clin. Embryol.* **13**, 33-41.