LifeGlobal
An IVFonline Company

U.S./Canada 800 -720 – 6375
International (+1) 519 - 826 - 5800
www.LifeGlobal.com

Instructions for the Use of global® total™ for Fertilization

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® total™ for Fertilization** for culture and conventional in-vitro fertilization of human oocytes.

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™ for Fertilization** 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: Human oocyte culture and fertilization

Catalogue Nos: LGTF-050 (50 ml), LGTF-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 oocyte, attached cumulus cells, and sperm.

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 use, 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™ for Fertilization**. 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™ for Fertilization**.

V. Instructions for Use

1. Prepare dishes for oocyte holding and/or fertilization, containing appropriate-sized droplets or larger volumes of **global® total™ for Fertilization** under oil, according to general laboratory practice.
2. 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. At the conclusion of the retrieval, dissect the oocytes to remove any degenerate and/or excess cumulus cells, blood and debris, and wash the oocytes, according to your standard laboratory procedures.
4. Transfer the oocytes into the **global® total™ for Fertilization** droplets in the oocyte-holding dish(es) (1-2 oocytes/ droplet).
5. Evaluate each oocyte according to your standard laboratory protocol.
6. Place the oocyte-holding dish(es) into a CO_2 incubator and culture for 3-6 hours; 3-4 hours if the majority of oocytes appear mature, up to 6 hours if the majority appear intermediate or immature.
7. Add sufficient sperm to each droplet of **global® total™ for Fertilization** in the fertilization dishes to produce the required sperm concentration.
8. Let the fertilization dishes sit for several minutes and then examine each droplet to ensure that the sperm concentration is appropriate.
9. Transfer the oocytes from the oocyte-holding dish(es) to the sperm-containing droplets in the fertilization dishes (1-2 oocytes/microdrop).
10. Place the fertilization dishes into a CO_2 incubator and culture, according to standard laboratory practice.
11. Evaluate the oocytes for evidence of fertilization, and wash and transfer them to embryo culture medium, according to your standard laboratory procedures.