



## Instructions for the Use of HTF

(Catalogue Numbers: GMHT-100, GMHT-250, GMHT-500)

### PRECAUTIONS AND WARNINGS

- 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 Instructions for Use, Precautions and Warnings, and be trained in the correct procedure before using the HTF for the Culture of cleavage-stage human embryos (Day 1-3).
- Not to be used for injection.
- Do not resterilize.
- 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
- HTF contains the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.
- To avoid problems with contamination, practice aseptic techniques.
- Discard unused medium within 7 days of opening. Do not use after expiry date.

### GENERAL INFORMATION

#### Indications for Use

Culture of cleavage-stage human embryos (Day 1 to Day 3).

#### Storage and Shelf Life

Store at 2-8°C and protected from light. Ten (10) weeks from the date of manufacture.

#### Composition

A bicarbonate-buffered medium replete with glucose, lactate, and pyruvate is required to support the growth and development of human embryos in vitro.

Sodium Chloride	Potassium Chloride	Calcium Chloride	Potassium Phosphate	Magnesium Sulfate
Sodium Bicarbonate	Glucose	Lactate Na Salt	Sodium Pyruvate	Phenol Red
Gentamicin Sulfate* (10 µg/ml)				

\*from therapeutic-grade source material

### QUALITY CONTROL SPECIFICATIONS

Assay (performed for each batch)	Specification
<b>Physicochemical Tests</b>	
pH (with 5% CO <sub>2</sub> )	7.2-7.4
Osmolality	280-292 mOsM
<b>Biological Tests</b>	
Endotoxin (LAL)	≤ 0.5 EU/ml
Sterility Test (bacterial and fungal screen, SAL 10 <sup>-3</sup> )	PASS
<b>Biological Assays</b>	
1-cell Mouse Embryo Assay (% expanded blastocysts at 96 h of culture)	≥ 80%



**Special Note on the CO<sub>2</sub> Concentration in the Incubator:** In most cases, a 5-7% concentration of CO<sub>2</sub> in the incubator will produce a pH of 7.2 to 7.4 in HTF. However, the exact concentration of CO<sub>2</sub> 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<sub>2</sub> that is required to produce a pH of 7.30 in HTF.

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## INSTRUCTIONS FOR USE

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The procedures described below have been found to be effective for the culture of human embryos. Every laboratory must define and optimize its own procedures.

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

Twenty-four (24) hours prior to the use of HTF for embryo culture, supplement the medium with either Human Serum Albumin (HSA) or LifeGlobal<sup>®</sup> Protein Supplement to achieve desired % (v/v) of protein supplementation.

### CO<sub>2</sub> and Temperature Equilibration

#### **Method 1: The day before embryo culture:**

1. Slowly gas the supplemented HTF in the 50 ml tissue culture flask for approximately 15 seconds with 5:5:90 Blood Gas Mixture using a sterile Pasteur pipette.
2. Allow the bubbles to rise to the neck of the flask.
3. Cap the culture flask tightly and stored in the refrigerator at 2-8°C. Over night the gas bubbles will disperse into the media, bringing the medium to the appropriate pH (7.2-7.4).
4. The next day, 1 hour prior to embryo culture, prepare microdrops of the gassed, supplemented HTF and place in the incubator for final CO<sub>2</sub> and temperature equilibration.

#### **Method 2: The day before embryo culture:**

1. Prepare microdrops of supplemented HTF in culture dishes, under oil. Make certain that the oil dishes have had 48 hours to equilibrate prior to making the microdroplets and/or use oil from a flask that has been equilibrated with culture medium in the incubator. Using equilibrated oil will insure that the pH of the medium is reached prior to the onset of embryo culture.
2. Place the culture dishes in the incubator overnight for CO<sub>2</sub> and temperature equilibration.

### Preparation of Microdrops for Culture of Embryos from Day 1 to Day 2 or Day 3

#### **Method 1: The day before embryo culture:**

1. Label the bottoms of an appropriate number of culture dishes with the patient's name.
2. Using a serological pipette, fill each dish with washed, room-temperature appropriate oil.
3. Place all dishes into the appropriate patient incubator for overnight equilibration.

#### **On the day of embryo culture (Day 1):**

1. Use a sterile pipette or tip, to underlay an appropriate number of 20–30 µl microdrops of supplemented HTF into each equilibrated oil dish.
2. Place all dishes into their inverted lids and place in the incubator for 3 to 4 hours.
3. Wash the zygotes and transfer them to the microdrops.
4. Place the dishes in the appropriate patient incubator for culture until Day 2 or Day 3.
5. Evaluate the embryos using your standard laboratory criteria and protocols.
















**Method 2: The day before embryo culture:**

1. Label the bottoms of an appropriate number of culture dishes with the patient's name.
2. Using a serological pipette, fill each dish with washed, room-temperature appropriate oil. Use a sterile pipette or tip, to underlay an appropriate number of 20–30 µl microdrops of supplemented HTF into each equilibrated oil dish.
3. Place all dishes in the incubator overnight equilibration.

**On the day of embryo culture (Day 1):**

1. Wash the zygotes and transfer them to the microdrops.
2. Place the dishes in the appropriate patient incubator for culture until Day 2 or Day 3.
3. Evaluate the embryos using your standard laboratory criteria and protocols.

**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	GS1 DataMatrix Barcode	Do Not Resterilize
					
European Conformance (notified body)	Do Not Use if Package is Damaged				