LifeGlobal®
GROUP

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Instructions for the Use of Washing mHTF w/ HEPES & Phenol Red

(Catalogue Numbers: LGFR-100, LGFR-500)

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 the Washing mHTF w/ HEPES & Phenol Red when handling gametes and embryos outside a CO_2 incubator.

3. Not to be used for injection.

4. Do not resterilize.

5. 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

6. Washing mHTF w/ HEPES & Phenol Red contains the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.

7. Washing mHTF w/ HEPES & Phenol Red contains only a low concentration of sodium bicarbonate and should not be gassed, or used for culture under CO_2.

8. To avoid problems with contamination, practice aseptic techniques and discard minimal amounts of excess medium remaining in the bottle.

9. Discard unused medium within 7 days of opening.

GENERAL INFORMATION

Indications for Use

For human oocyte retrieval and washing outside of a CO_2 incubator.

Storage and Shelf Life

Store at 2-8°C and protected from light. Fourteen (14) weeks from the date of manufacture.

Composition

A HEPES-buffered medium is required when gametes and embryos are handled outside of a CO_2 incubator.

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

*from therapeutic-grade source material

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QUALITY CONTROL SPECIFICATIONS

Assay (performed for each batch)	Specification
Physicochemical Tests	
pH	7.2-7.4
Osmolality	280-292 mOsM
Biological Tests	
Endotoxin (LAL)	≤ 0.5 EU/ml
Sterility Test (bacterial and fungal screen, SAL 10^{-3})	PASS
Biological Assays	
1-cell Mouse Embryo Assay (% expanded blastocysts at 96 h of culture after 1 h exposure)	≥ 80%

INSTRUCTIONS FOR USE

Media Required for Oocyte Retrieval

1. **Needle-Wash Medium:** Use Washing mHTF w/ HEPES & Phenol Red to wash the collection needles prior to oocyte retrieval.

2. **Follicle-Flush Medium:** Use Washing mHTF w/ HEPES & Phenol Red to flush the follicles, if appropriate.

3. **Oocyte-Wash Medium:** Use Washing mHTF w/ HEPES & Phenol Red supplemented with protein to wash the oocytes after retrieval.

4. **Oocyte Culture Medium:** Use global® for Fertilization supplemented with protein for the final wash and culture of the oocytes prior to fertilization.

The procedures described below have been found to be effective for human oocyte retrieval and washing outside of a CO_2 incubator. 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.

1. The day before oocyte retrieval, aliquot appropriate amount of oil into a 14 ml Falcon tube, and place it in a warming block (36-38°C) to equilibrate overnight.

2. Twenty-four (24) hours prior to the use of Washing mHTF w/ HEPES & Phenol Red, prepare oocyte-wash medium by supplementing the medium with either Human Serum Albumin (HSA) or LifeGlobal® Protein Supplement to achieve desired % (v/v) of protein supplementation. Store overnight at 2-8°C.

3. Place a tube of oocyte-wash for each patient into the warming block.

4. Place 10-12 100 mm Falcon dishes and 1-2 60 mm Falcon dishes on the warming surface of the laminar flow hood.

5. Prepare dishes containing oocyte culture medium for final oocyte wash and culture.

6. Just prior to oocyte retrieval, prepare the initial oocyte wash dish by pouring of oocyte-wash medium into a warmed 60 mm Falcon dishes. Cover the oocyte-wash medium with pre-equilibrated oil and place the dish on the warming surface of the laminar flow hood.

7. During oocyte retrieval, search for the oocytes under a dissecting microscope. Using a sterile pipette, transfer each oocyte into the initial wash dish containing oocyte-wash medium.

8. Dissect each oocyte using two 25 g 11/2” needles to remove the degenerated cumulus cells, debris and blood.

9. Transfer all oocytes to a dish of the oocyte culture medium for final washing. Pipette all the oocytes a few times to remove traces of oocyte-wash medium.

10. At the conclusion of oocyte retrieval, transfer each dissected oocyte into fresh culture media, according to your standard laboratory practice, and place in the incubator until fertilization or ICSI.

SYMBOLS

STERILE A	**RX Only**	REF	LOT		
Sterile Using Aseptic Processing Techniques	By Prescription Only	Catalogue Number	Batch Code	Consult Instructions For Use	Manufacturer
		EC REP			
Keep Away From Sunlight	Temperature Limitation	Authorized Representative in the European Community	Use By	GS1 DataMatrix Barcode	Do Not Resterilize
CE 2797					
European Conformance (notified body)	Do Not Use if Package is Damaged				