

SYNBIOS[®] CULTURE MEDIUM

GENERAL INFORMATION ON USE

SYNBIOS[®] Culture Medium is a fertilization & embryo culture formulation for fertilization of oocytes and culture of embryos on continuous basis to days 2 or 3 and up to blastocyst stage in a 6% carbon dioxide atmosphere at sea level. **There are two types, one with HSA, while the other is a synthetic protein-free medium with surfactant macromolecules. Both are complete media that need no other additives and are ready-to-use.**

MATERIALS SUPPLIED

50 mL SYNBIOS[®] Culture Medium with Phenol Red with Gentamicin and HSA (Ref: EM10050)

50 mL SYNBIOS[®] Culture Medium with Phenol Red with Gentamicin without HSA (Ref: EM10050.SYN)

MATERIALS REQUIRED BUT NOT SUPPLIED

Test tubes & culture dishes; Oocyte and embryo handling pipettes; Laminar flow/Biological hoods, Stereo & Inverted Microscopes; Incubators, centrifuge, and other ART Lab equipment. Others include: SYNBIOS[®] Mineral Oil; Flush, Gamete, Sperm, PVP, and Hyaluronidase media for cIVF / ICSI.

SPECIFICATIONS AND QUALITY CONTROL

SYNBIOS[®] Culture Medium comes with and without human serum albumin (HSA) as described previously. It contains carbohydrates, sugars, physiological salts, amino acids, vitamins, sodium pyruvate, sodium lactate, osmolytes such EDTA, and sodium bicarbonate. All products contain extremely minute quantities of Gentamicin Sulfate in the order of 1.5 to 4.0mg per Liter, and/or Phenol Red for pH balance. Quality is assured through testing for sterility, pH, osmolality, endotoxin <0.05 EU/mL (USP, PH Eur.) and Mouse Embryo Assay. A Certificate of Analysis for each batch is available on request.

STORAGE AND CONSERVATION

Product must be stored in original package between 2-8°C. It must not be aliquoted into smaller containers for storage. Once removed from container, discard excess medium. Do not freeze. **IMPORTANT:** It is not possible to sterilize HSA with 100% certainty (Truyen et al., 1995) thus HSA must be treated as potentially infectious. **Do not use this product if:**

- The medium appears cloudy or shows signs of microbial contamination.
- The product has expired.
- The seal of product is broken.
- The container is damaged.
- Cold chain has been broken during transport and handling.

CAUTION

Culture medium exposed to the elements or above 8°C for >8 hours may be unfit for use for human ART treatment due to possible formation of toxic free radicals and products of putrefaction.

DISCLAIMER:

Every effort has been taken to ensure quality of the product. We have no control over the products during transport. Shippers are aware of the cold-chain protocol. Nevertheless product could be damaged for any reason during transport (e.g. inspection by Border Security). End users must ensure products received are in good condition by internal QC/QA prior to use.

WARNINGS AND PRECAUTIONS

This product must be used only by laboratory personnel competent in laboratory human Assisted Reproduction Technology (ART). All human and organic material is potentially infectious; including this product, if it contains HSA. All specimens must be handled as capable of transmitting harmful viral or prion diseases or hitherto unknown pathogenic agents. Wear protective garments. Strict aseptic techniques must be employed to avoid contamination. Medium may contain antibiotic Gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.

INSTRUCTIONS FOR USE

1. The SYNBIOS[®] Culture Medium must be pre-equilibrated for a minimum of 6 hours in 6% CO₂ at 37°C in culture dishes under SYNBIOS[®] Mineral Oil or in open 4-well dishes or for 18 hours in culture tubes prior to use.
2. Perform the ART procedure (oocyte collection, insemination, fertilization check, embryo culture and transfer) as per your standard operating procedures (SOP) using the SYNBIOS[®] Culture and Handling Media products.
3. The SYNBIOS[®] Culture Medium can be used for (a) holding oocytes until insemination or ICSI; (b) holding harvested sperm prior to insemination or ICSI; (c) fertilization of oocytes and (d) culture of fertilized oocytes (zygotes) from day 1 up to blastocyst, hatching and hatched blastocyst stages on a continuous basis as per the standard operating procedures (SOP) of your laboratory.
4. For blastocyst culture, the culture milieu is augmented with fresh SYNBIOS[®] Culture Medium on day 3. This augments embryos with more nutrients for growth and conserves paracrine and autocrine factors in culture (Paria and Dey, 1990; Ali 2004). This step is not needed for uninterrupted culture procedures.

5. Protocol for Insemination and Culture.

The oocytes are identified and recovered from follicular aspirates, then washed in SYNBIOS® Gamete Medium held at 37°C in labelled 4-well dishes (0.8ml per well and 2ml in reservoir). Washed oocytes are transferred to the SYNBIOS® Culture Medium held at 37°C under an atmosphere of 6%CO₂ in labelled 4-well dishes. Perform insemination within 4 hours of oocyte collection at ≥38 to ≤40hrs post-hCG in SYNBIOS® Culture Medium at 37°C under an atmosphere of 6%CO₂, 5%O₂, 89%N₂ or 6%CO₂ in air.

6. Insemination by cIVF: Adjust the spermatozoa number to 100,000 motile sperm per ml culture medium per oocyte or 20,000 motile sperm per 20ul micro-drop culture per oocyte or use your own protocol. Perform fertilization check by 16 to 22 hrs.

7. Intracytoplasmic Injection (ICSI)

Expose oocytes to equilibrated SYNBIOS® Hyaluronidase for 30 seconds only in well-1 of a 4-well dish held at 37°C under an atmosphere of 6%CO₂ in labelled 4-well dish with constant pipetting to disperse the cumulus- corona cells. Wells 2 to 4 contains SYNBIOS® Culture Medium. Transfer the exposed oocytes to well 2 and incubate (6%CO₂) for 5mins. Then pipette the eggs individually with 170 µm bore stripping pipette followed by 155 µm bore pipette Until all or >95% of the cumulus-corona have been

stripped away. The dish must not be out of incubator for more than ≤3 mins. If unable to complete denuding within 3 mins replace dish in incubator for 10 mins and continue with denuding after 10 mins. Wash the stripped/denuded eggs in well 3 and place the washed oocytes in well 4. The eggs are incubated further at 37°C under an atmosphere of 6%CO₂ until insemination by ICSI which must be performed within <40hrs after hCG administration.

9. Detection of Fertilization and Culture of Zygotes

Perform fertilization check after overnight incubation of 16 hours and within <22 hours post insemination. Culture normal 2PN zygotes singly or in groups of 5 zygotes per culture droplet, or as per your SOP, in SYNBIOS® Culture Medium held at 37°C under an atmosphere of 6%CO₂. Abnormal zygotes are likewise cultured in separate droplets or discarded as per SOP. Return dish to incubator under an atmosphere of 6% CO₂ in air and culture until day of ET (days 2,3 or 5/6).

10. Protocol for Day 3 Medium Augmentation

If you are using 10ul micro-drops for culture then add to it another 10ul of fresh culture medium on day 3. If your micro-drop is 20 or 30ul then remove 10 or 15ul of medium from the said micro-drop and replenish it with 10 or 15ul respectively with fresh culture medium. If you are using the ultra micro-drop culture system, add fresh culture medium into the ultra micro-drops

three (x3) times its original volume (for instance if the original volume of your ultra micro-drop was 2ul, then to this add 6ul of fresh culture medium).

11. Culture of Embryos to Days 2, 3, 5-7

Zygotes are cultured in SYNBIOS® Culture Medium at 37°C under an atmosphere of 6%CO₂, 5%O₂, 89%N₂ or 6%CO₂ in air until day 2 if embryo transfer (ET) is to be performed on day 2, up till day 3 if ET is to be performed on day 3 or up to day5/6 and up to day 7 if blastocyst transfers are desired. Quality blastocysts should appear by day 5 and as early as late day 4.

Good Laboratory Practices

ART Lab personnel are urged to adhere to Good Laboratory Practices (GLP) for optimizing the treatment outcome. Dishes must not be out of incubator for more than 3 mins at any one time.

Note: This product is classified as a medical device. US Federal Law restricts its sale by or on order of a physician (Rx only).

Custom-produced under GMP quality conditions

For ANDROCRYOGENICS, Malaysia

www.synbiosmedia.com

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May 2020

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**SYNBIOS® EMBRYO
CULTURE MEDIUM**

20 Years of Research

Ensures Optimal Performance

SYNBIOS® MEDIA
Safety. Performance. Innovation

GMP-Manufactured

Embryo culture medium formulation for fertilization and single-medium continuous embryo culture to blastocyst stage

Ref: 50 mL EM10050

Ref: 50 mL EM10050.SYN

SYNBIOS® EMBRYO CULTURE MEDIUM is sterilized by sterile filtration and comes with (Ref: EM10050) and without HSA (Ref: EM10050.SYN).