



SYNBIOS® HYPO OSMOTIC MEDIUM

GENERAL INFORMATION ON USE

SYNBIOS® HYPO OSMOTIC SPERM TAIL SWELLING Medium is a formulation for selecting potential live and possible viable spermatozoa in instances when available sperm are immotile and no motile spermatozoa can be obtained for the ICSI procedure. 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

5 mL & 10mL SYNBIOS® Hypo osmotic Medium with Phenol Red with Gentamicin and HSA (Ref: SH10005 & SH10010)

5 & 10 mL SYNBIOS® Hypo osmotic Medium with Phenol Red with Gentamicin without HSA (Ref: SH10005.SYN & SH10010.SYN)

MATERIALS REQUIRED BUT NOT SUPPLIED

Test tubes, culture dishes; pipettes; Laminar flow hoods, Stereo & Inverted Microscopes; Incubators, centrifuge, etc. Others include: SYNBIOS® Mineral Oil; Flush, Gamete, Sperm, PVP, and Hyaluronidase media for ICSI.

SPECIFICATIONS AND QUALITY CONTROL

SYNBIOS® HYPO OSMOTIC SPERM TAIL SWELLING 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. Contains 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. 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

SYNBIOS® HYPO OSMOTIC SPERM TAIL SWELLING 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. During the ICSI procedure if no motile sperm or no sperm movement is present in the preparation, the SYNBIOS® HYPO OSMOTIC SPERM TAIL SWELLING Medium, a hypo-osmotic solution, could be used to identify live/viable immotile sperm for ICSI.
2. Principle of this test is the sperm tail will swell when exposed to the SYNBIOS® HYPO OSMOTIC SPERM TAIL SWELLING Medium if the sperm is alive/viable. **ADHERE TO ASEPTIC TECHNIQUE.**
3. Place a 5µl droplet of SYNBIOS® HYPO OSMOTIC SPERM TAIL SWELLING Medium near the PVP spread during preparation of the ICSI dish. The hypoosmotic medium can be spread flat but not too thin to avoid evaporation. Cover with oil as soon as possible to avoid media drying.
4. A single non-motile but morphologically normal sperm is picked up, head first, using the ICSI injection pipette from the PVP spread or gamete media containing washed immotile sperm.
5. The injection pipette is moved to the 5µl droplet of the hypo-osmotic solution. The tail of the spermatozoon is slowly brought into contact with the hypo-osmotic solution, without releasing the entire spermatozoon into it.
6. If the spermatozoon is viable or alive its tail may swell and curl-up. Before complete swelling or curling

of the tail occurs, the spermatozoon is sucked back into the injection pipette, then the injection pipette is withdrawn.

7. The spermatozoon is then washed in PVP 7 - 8 times, before finally being injected into the oocyte.

8. Please note a tail injury must be inflicted on the spermatozoon that has been selected for injection, even if non-motile prior to injection.

9. The spermatozoon selected for injection, as far as possible, should be morphologically normal so that better quality embryos can be generated giving rise to higher clinical pregnancy rates.

10. Injected oocytes are cultured in equilibrated and warmed SYNBIOS® EMBRYO CULTURE Medium as per standard protocol.

11. **Caution:** There is no guarantee this technique will yield fertilized oocytes. The hypoosmotic medium could be potentially harmful to the oocyte. Please ensure the oocyte is not exposed to the hypoosmotic medium.

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 and culture until day of ET (days 2,3 or 5/6).

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 day 5/6 and up to day 7 if blastocyst transfers are desired. Quality blastocysts should appear by day 5 and as early as late day 4.

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).

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**

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**SYNBIOS® HYPO
OSMOTIC 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: 5 mL SH10005 with HSA

Ref: 5 mL SH10005.SYN without HSA

Ref: 10 mL SH10010 with HSA

Ref: 10 mL SH10010.SYN without HSA

SYNBIOS® EMBRYO CULTURE MEDIUM is sterilized by sterile filtration and comes with and without HSA.