

**SYNBIOS®VITRIFICATION WARMING KITS**

**For warming and rehydration of cryopreserved (vitrified) human oocytes, and embryos up to blastocyst stage**

**COMPOSITION**

Media are SYNBIOS®- Gamete medium based and contains HEPES, with or without human serum albumin (HSA;20 g/liter), and sucrose. Antibiotics is not present.

**MATERIAL INCLUDED WITH THE KIT ARE INDICATED BELOW:**

One kit will provide sufficient medium for approximately 4-6 procedures.

**VM10000 Warming kit** contains vial each of the following media and cryoprotectant solutions:

VW10001 Warming solution 1 (WS1)	4.0mL x 2
VW10002 Warming solution 2 (WS2)	4.0mL
VW10003 Warming solution 2 (WS3)	4.0mL

**MATERIAL NOT INCLUDED WITH THE KIT**

- Dish, shallow 4-well, sterile, non-toxic;
- Dish 1-well sterile, non-toxic (ET dish)
- Cooling tank with liquid nitrogen
- Embryo handling pipets
- 12 inch Forceps
- Laminar flow hood
- Zoom stereo microscope
- Lab Timer
- SYNBIOS EMBRYO CULTURE MEDIUM

**SYNBIOS® VITRIFICATION WARMING** kit is compatible for use with SYNBIOS vitrification cooling kit, SYNBIOS Culture medium and SYNBIOS Gamete/Flushing media to culture, wash oocytes and embryos before vitrification and after warming.

**PRODUCT SPECIFICATIONS**

- Chemical composition
- pH: 7.20 – 7.40
- Osmolality (mOsm/kg):Pre-incubation med: 270-290
- Sterility: Sterile (SAL 10-3)
- Endotoxins: < 0.25 EU/ml
- Mouse Embryo Assay (blastocysts after 120h) ≥ 80%
- Use of Ph Eur or USP grade products if applicable
- Certificate of analysis and MSDS are available upon request

**STORAGE INSTRUCTIONS**

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. Once opened the vial can be used for 7 days provided aseptic technique is adhered to at all times and it is stored at 2-8°C. Do not use after expiry date.

**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. •The product must not be used if any of the media supplied is cloudy and also do not use the product if the seal has been removed or is defective.

**IMPORTANT:** It is not possible to sterilize HSA with 100% certainty (Truyen et al., 1995) thus HSA must

be treated as potentially infectious. ART Lab personnel are urged to wear personnel protective apparel including goggles for their safety. Lab Personnel must adhere to Good Laboratory Practices (GLP) for optimizing outcome and to avoid mishaps. **CAUTION:**All media 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.

**INSTRUCTIONS FOR USE**

The product must be brought to room temperature before use (-22°C). **Read instructions carefully before commencing on the procedure** Practice is needed. The entire procedure must be performed aseptically.

**A. WARMING AND REHYDRATION OF VITRIFIED OOCYTES**

**I. Warming procedure for Micro devices**

[The word "Thawing" has no place in the vitrification procedure because vitrification is an ice-free technique whereas the word "thawing" implies ice-formation occurred which is erroneous so we shall use the word "warming" instead. The vitrified-warmed oocyte will be in a state of **severe physical trauma** so it must be handled as gently as possible throughout the procedure especially when pipetting otherwise it may not survive the procedure.

1. Use a 1-well dish for step 1 of the procedure. Place 0.65ml of WS1 in the center well and leave it for 30-45mins it to warm to 37°C in an ordinary incubator without CO<sub>2</sub>.
2. Take a 4-well dish and place in it 400ul of WS1 in well-1, 400ul of WS2 in well-2, 400ul of WS3 in well-3. Overlay these 3 wells with 400ul of mineral oil.
3. Place 0.7ml mineral oil in well 4. Put its lid back on and keep in at room temperature.
4. Identify the microdevice that is to be warmed. Place it in a small cryo tank filled with liquid nitrogen. This step must be witnessed to ensure the correct device was retrieved.

5. Without exposing the device to the air for any more than 1 second open and place the part of the device holding the oocytes into WS1 held in the 1-well dish at 37°C. This must be performed under microscope.
6. This step must not take any more than 1, maximum 2 seconds, preferably 1 second. Within 1 minute identify the oocytes and at exactly 1 minute transfer the oocytes to WS1 held in well 1 of the 4-well dish that is at room temperature 22-24°C.
7. Leave it in well-1 of the 4-well dish for 6 minutes. Continue to hold the 4-well dish at room temperature.
8. At 6 minutes transfer the oocytes to WS2 held in well 2 and hold it in this well for 3 mins.
9. Midway, that is about 1.30 mins during this 3 minute waiting time place a place a 30ul droplet of pre-equilibrated SYNBIOS culture medium underlaid over the oil in well-4.
10. Importantly, although equilibrated with CO<sub>2</sub> it must be at room temperature. The culture medium droplet must only be made soon after the oocytes have been transferred to well 2. Alternatively the culture medium can be prepared in a separate dish to avoid mistakes.
11. These steps are shown in the chart 1 below:

Chart 1: Warming Protocol for Micro Devices

Dish/Well	1-well Dish 37°C	4-well dish well 1	4-well dish well 2	4-well dish well 3	4-well dish well 4
MEDIA	WS1 0.8ml	WS1 400ul	WS2 400ul	WS3 400ul	CM 20ul
TIME	1 min	6 min	3 mins	1 min Wash	Return dish to CO2 incubat or

12. At end of the 3 minutes transfer the oocytes to well WS3 in well 3. Leave it in WS3/well-3 for 30-45 seconds. Gently swirl the WS3 medium with pipet to wash off WS2 without touching the oocytes.
13. After the 1 minute of wash gently transfer the oocytes to the culture medium held in a droplet under oil in well-4 and soon after this place the dish in CO<sub>2</sub>

incubator for 3 hours.  
14. ICSI can be performed after 3 hours of incubation.

**II. WARMING AND REHYDRATION OF OOCYTES VITRIFIED IN STRAW.**

1. The straw warming procedure is identical to that of micro devices except that the warming.
2. The straw shall be warmed in a water bath held at 25°C for 5 to 6 seconds with a gentle swirling motion.
3. The contents of the straw must be quickly emptied into the center well of the 1-well dish held at room temperature (not 37°C). This step is critical, it must be performed under microscope so that the oocytes can be visualized as it comes out of the straw. Pre-plan, improvise if necessary and perform this step carefully.
4. The oocytes must be identified rapidly and transferred at 1 minute to well-1 of the 4-well dish containing WS1 medium and held in it for 6 minutes .
5. The rest of the procedure will be identical to that described for the micro device in the previous section, briefly, the oocyte is transferred to Well-2 for 3 mins in WS2, then 1 minute in WS3, and then moved to the culture medium. The dish is then placed in the CO<sub>2</sub> incubator for 3 hours after which the oocytes can be inseminated by ICSI. (See Chart 2 below)

Chart 2: Warming Protocol for Straw Device @ RT

Dish/ Well	1-well Dish 22°C	4-well dish well 1	4-well dish well 2	4-well dish well 3	4-well dish well 4
MEDIA	WS1 0.8ml	WS1 400ul	WS2 400ul	WS3 400ul	CM 20ul u/oil
TIME	1 min	6 min	3 mins	1 mins Wash	Return dish to CO <sub>2</sub> incubat or

**B. WARMING AND REHYDRATION OF EMBRYOS OF ALL EMBRYO DEVELOPMENTAL STAGES**

The protocol for the warming and rehydration of

vitrified zygotes and all other developmental stages of the embryo vitrified in micro devices or straws are identical to that described for the oocyte in Sections 1 (I) & (II) respectively. The embryos will be ready for embryo transfer or for any other procedure after 3 hours of incubation in the CO<sub>2</sub> incubator.

**AVOID DISEASE TRANSMISSION:** For oocytes and all stages of the embryo the dish used for warming and rehydration/ dilution can only be used for one patient. This is to avoid disease transmission and to comply with GLP/GCP. Always use new dish for the next patient.

**BACKGROUND**

Vitrification was previously abandoned in the 1960's due to toxicity issues but research and development efforts by a number of pioneering groups the world over beginning in mid-1980's through the mid 2000's refined the vitrification technique such that its toxicity and safety issues have been largely overcome. These developments have transformed cryopreservation completely with vitrification displacing controlled-rate slow freezing. RCTs have noted vitrification is superior to conventional controlled-rate slow-freezing technique with regard to clinical outcomes and cryosurvival rates for oocytes, cleavage-stage embryos and blastocysts. Vitrification techniques have achieved high level efficacy that fertilization, generation of quality embryos and on-going pregnancy rates following oocyte vitrification are comparable to that of fresh oocytes. Vitrified oocyte survival rates were higher than that obtained after slow-cooling. Vitrification has now completely overshadowed other techniques of cryopreservation for oocytes and embryos. Although more time is needed to determine long term biosafety of vitrification with a greater level of certainty but data available to

date did not cast any fear of significant overt adverse outcome. Vitrification remains the only viable option available after slow, rapid and ultra-rapid freezing techniques have been proven to be less efficacious. For more information refer to practice guidelines of professional learned societies and directives of regulatory authorities.

**REFERENCES**

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3. Practice Committee of the ASRM; SART. Mature oocyte cryopreservation: a guide. Fertil Steril. 2013;99(1):37-43.
4. Rienzi et al. (2017). Oocyte, embryo and blastocyst cryopreservation in ART. Human Reprod Update 23(2):139-155.
5. Cai et al. (2018), Open versus closed vitrification system of human oocytes and embryos. Reprod Biol Endocrinol 1:123

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

Custom-manufactured for ANDROCRYOGENICS, Malaysia  
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**SYNBIOS® WARMING AND REHYDRATION KIT**

**20 Years of Research**

**Ensures Optimal Performance**

**SYNBIOS® MEDIA**  
Safety. Performance. Innovation

**GMP-Manufactured**

Human oocyte and embryo cryopreservation

Ref: VW10000 Warming Kit with HSA

Ref: VW10000.SYN Warming Kit without HSA

SYNBIOS® VITRIFICATION KIT is sterilized by sterile filtration. Comes with and without HSA.