

SYNBIOS® MEDIA
Safety. Performance. Innovation

SYNBIOS®VITRIFICATION COOLING KITS

for cryopreservation of 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), Ethylene Glycol (EG) 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.

VC10000 Cooling kit contains vials of the following media and cryoprotectant solutions:

- VC10001 Vitrif Preincubation medium (Pre) 1.5mL
- VC10002 Vitrif cooling solution 1 (ES1) 1.5mL
- VC10003 Vitrif cooling solution 2 (ES2) 1.5mL
- VC10004 Vitrif cooling solution 3 (VS) 1.5mL x 2

MATERIAL NOT INCLUDED WITH THE KIT

- Dishes, shallow 5-well, sterile, non-toxic;
- Cooling tank with liquid nitrogen
- Embryo handling pipets
- 12 inch Forceps
- Vitrification vehicle or device (e.g. Fibreplug and sleeves (Cryologic); Cryotop (Hunter Scientific); HSV straws (Cryo Bio Systems) or any other device type, as per your protocol
- Laminar flow hood

- Zoom Stereo Microscope,
- Lab timer
- Hand-held micro heat sealer

SYNBIOS®VITRIFICATION Cooling kit is compatible for use with **SYNBIOS vitrification warming kit**, SYNBIOS Culture medium and SYNBIOS Gamete/Flushing medium 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.** The entire procedure must be performed aseptically. Mock runs and practice is needed before actual procedure.

1. VITRIFICATION OF OOCYTES

Cooling procedure: Use a shallow 5-well sterile dish.

The first well is filled with 350µl of Pre-incubation medium, likewise the second with Cooling ES1, the third with Cooling E2 and the next two wells will contain VS in both wells. Prepare dish on the spot. **IMPORTANT:** Fill wells-4 and 5 with VS just two, preferably one minute before transferring the oocytes into it. Reason: to avoid hygroscopic effects or humidification that will reduce the osmolality of the VS which is detrimental leading to ice-formation.

PREPAREDNESS: Keep ready at hand for fast and easy access the required numbers of vitrification vehicles or devices and their parts in sterile condition, forceps, heat sealer, etc. Each vehicle can accommodate only 2-3 oocytes loaded in no more than 1µl of VS for micro vehicles. (Read the instructions for use of vehicles carefully before use).

Number of cycles per dish set-up: One run per set-up is the best way to go due to hygroscopicity risks mentioned previously. However up to 3 runs can be performed with the same dish set-up for the same patient ONLY if performed rapidly, provided the last well of VS is replaced just before use each time.

COOLING PROTOCOL: Oocytes are sequentially exposed to the cryo media shown in Chart 1 below held in wells 1 to 5 at room temperature 22-26°C. **CRITICAL:** When transferring oocytes between the wells ensure excess media from one well is not transferred to medium in next well during oocyte transfer to avoid diluting the next medium in the sequence. Otherwise the procedure will fail. Timing of the procedure is crucial. **IMPORTANT:** First 3 steps are in minutes and last two in seconds

Chart 1: Protocol for oocyte vitrification

Well	1	2	3	4	5
MEDIA	PRE	ES1	ES2	VS (1)	VS (2)
TIME	2 min	4 min	8-11 min	10 second 5	50 second 5

***ES2:** Equilibrate in ES2 until oocyte has re-expanded to original volume, usually in 8 mins. **Note:** maximum time in ES2 is 11 mins. Remove all trace of ES2 in pipet before transferring oocytes to VS(1).

***VS:** Once oocyte is in VS(1) swirl the media solution gently with pipet to promote mixing. Quickly pick up fresh VS into the pipet from next well. Then pick up the oocytes and transfer to VS(2) and mix well.

CRITICAL: The entire duration of 10+50 = 60 seconds in the last two VS wells must NOT EXCEED 60 seconds. This 60 second duration also includes loading under microscope oocytes onto or into the vitrification vehicles depending on the type of vehicle. When the oocytes are placed in the vehicle the volume of VS medium deposited together with the oocytes must not exceed 1µl for micro vehicles. If straws are used follow appropriate protocol. Quickly seal or lock the vehicle and plunge it into liquid nitrogen before 60 seconds. This is common to most vitrification method. VITRIFICATION TECHNIQUES NEEDS CONSIDERABLE PRACTICE.

2. VITRIFICATION OF DAY 1 ZYGOTES AND CLEAVAGE STAGE (DAYS 2 AND 3) EMBRYOS

1. The protocol for the vitrification of zygotes, and cleavage stage days 2 and 3 embryos are identical to that of oocytes except for exposure durations in ES1 and ES2 equilibration media solutions given in chart.
2. When the embryo has assumed its original volume in ES2 the embryos are moved to VS (1). Do not exceed 7 mins in ES2 media.

Chart 2: Protocol for cleavage stage embryos

Well	1	2	3	4	5
MEDIA	PRE	ES1	ES2	VS (1)	VS (2)
TIME	2 min	5 min	5-7 min	10 second s	50 second s

Each vehicle can accommodate only 2, maximum 3 embryos loaded in no more than 1µl for micro vehicles. If using straws this rule does not apply. Use the appropriate protocol for straws provided by the manufacturer.

3. VITRIFICATION OF DAYS 5 & 6 BLASTOCYST STAGE EMBRYOS

1. The protocol for the vitrification of Days 5 and 6 blastocyst stages are identical to that of oocytes.
2. For day 4 morula and early blastocysts follow the same protocol as blastocysts.
3. Hatching and hatched blastocysts (including Day 7 late developing blastocyst) could also be vitrified using this protocol but survival may be compromised for a number of reasons or if not very carefully handled.

IMPORTANT: if your SOP requires collapsing blastocysts manually by microinjection or any other method prior to vitrification you may perform it before exposure to the cryo media shown in the chart 3.

Blastocysts are exposed sequentially as shown in Chart 3:-

Chart 3: Protocol for blastocyst-stage embryos

WELL	1	2	3	4	5
MEDIA	PRE	ES1	ES2	VS (1)	VS (2)
TIME	2 min	4 min	8-11 min	10 second s	50 second s

AVOID DISEASE TRANSMISSION: For oocytes and all stages of the embryos the dish used for equilibration and exposure to VS can only be used for one patient. This is to avoid disease transmission and to comply with GLP/GCP. Always use a new dish for the next patient.

VITRIFICATION USING STRAWS

- The straw is filled with VS and the plug-end heat-sealed. It is held at room temperature.
- After the exposure to cryo media the oocytes/embryos are transferred into the straw through the open-end of the straw using a denuding or embryo handling pipet (145-170 microns) depending on the diameter of the specimen.
- The oocytes/embryos must be deposited well into the straw to rest onto its inner surface. performed under zoom stereo microscope.
- The transfer into the straw must be performed within the 60 seconds of exposure of blastocyst in VS.
- Heat-seal the open end and plunge the straw into liquid nitrogen using a 12-inch forceps holding the plug-end of straw keeping it below surface of liquid nitrogen with a gentle swirling motion until the crackling sound ceases. Let straw drop to the bottom.
- Transfer straw into goblet without taking straw out of liquid nitrogen. The goblet was previously placed in the same liquid nitrogen chamber for cooling equilibration.
- Store Goblet and specimen in specimen-storage cryo tank under liquid nitrogen.

References

- Ali, J. (1996) Developmental competence of unipronuclear and triploid day-2 human embryos after vitrification with VS14. Med. Sci. Res. (UK) 24:377-378 (available on request)

2. Practice Committee of the ASRM; SART. Mature oocyte cryopreservation: a guide. Fertil Steril. 2013;99(1):37-43.

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 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.

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
www.synbiosmedia.com

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May 2020

SYNBIOS® VITRIFICATION COOLING KITS

20 Years of Research

Ensures Optimal Performance

SYNBIOS® MEDIA
Safety. Performance. Innovation

GMP-Manufactured

Human oocyte and embryo cryopreservation

Ref: VC10000 Cooling Kit with HSA
Ref: VC10000.SYN Cooling Kit without HSA

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

