VitriBlast™ Kit

Intended Use

For vitrification of human blastocysts.

Caution

- Federal Law restricts this device to sale by or on the order of a physician or practitioner trained in its use.
- The user should read and understand the Directions for Use, Warnings and Precautions, and be trained in the correct procedure before using the Nidacon Kits for vitrification of human blastocysts.
- All blood products should be treated as potentially infectious . Source material from which this product was derived was found negative when tested for antibodies to HIV, HBc, HCV and HTLV I/II and non-reactive for HbsAg, HCV RNA and HIV-1 RNA and syphilis. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents
- Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation/removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens.

  There are no reports of proven virus transmissions with albumin manufactured to European Pharmacopoeia specifications by established processes.

  It is strongly recommended that every time VitriBlast™ is used for a patient, the name and batch number of the product are recorded in order to maintain a link between the patient and the batch of the product.

Warnings

- The long term safety of blastocyst vitrification on children born following this method of embryo cryopreservation is unknown
- The long term safety of blastocyst collapse on children born following this procedure has not been established

Precautions

- Use aseptic procedures at all times
- Do not use any vial or solution that shows evidence of particulate matter or cloudiness
- Do not use contents if tamper-evident seal is broken or if scew cap accidently comes in contact with unsterile surfaces
- VitriBlast™ Kit contains DMSO which is a highly pentetrable substance. A material safety data sheet is available from the distributor or manufacturer (see nidacon.com)
- VitriBlast™ Kit contains Ethylene glycol which is toxic when ingested.
- Please check for regulatory compliance governing the use of ART products in your country
- Do not re-use. Reuse may result in biological contamination and/or property changes in the product.

Components

Sodium chloride	Purified Water
Potassium chloride	Sodium pyruvate
Magnesium sulphate	EDTA
Potassium dihydrogen phosphate	HEPES
Sodium bicarbonate	Sucrose
hSA Human serum albumin	Ethylene glycol
Calcium lactate	DMSO
Glucose	Ficoll

Performance Characteristics

pH	7.20-7.50
Endotoxin levels	<0.5 EU/mL
MEA Reexpanded blastocysts after exposure	>80%
Sterile filtered	SAL 10^{-3}

Product Description

All VitriBlast™ Kit and ThermoBlast™ Kit solutions contain a modified HEPES buffered HTF medium. VitriBlast, solution 2, will after inclusion of additives also contain DMSO 7.5% and Ethyleneglycol 7.5%. VitriBlast™, solution 3, in addition includes Ficoll 0.14 mM, Sucrose 0.67 M and will after inclusion of additives also contain DMSO 15% and Ethyleneglycol 15% (DMSO and Ethyleneglycol are included as additives solely in the VitriBlast™ Kit). ThermoBlast™, solution 4, in addition contains Sucrose 0.5 M and solution 5 contains Sucrose 0.25 M

Protein Supplement

VitriBlast™ Kit contains the component human serum albumin (hSA)

Storage and Stability

Store the unopened bottles at 2 to 30ºC and avoid temperatures above or below these values. Under these conditions VitriBlast™ Kit has a shelf-life of 12 months. The expiry date is shown on both bottles and cartons

No claims are made regarding the shelf-life of VitriBlast™ Kit in opened vials

No antibiotics, unstable additives or preservatives have been added by the manufacturer to VitriBlast™ Kit

Ordering Information

Volume	**Article No.**
3x10 mL	VBK-010

www.nidacon.com

For further technical information or assistance, please contact your distributor or the manufacturer

Manufacturer:
Nidacon, Flöjelbergsgatan 16 B, SE-431 37 Mölndal, Sweden
Tel: +46-31-703 06 30, Fax: +46-31-40 54 15
E-mail: contact@nidacon.com, www.nidacon.com

Nidacon

INS-VBK-EC18

Reagents and Equipment

- VitriBlast™ kit
- Device for vitrification
- Inverted microscope
- Culture dishes (NUNC 4-well)
- Liqud nitrogen reservoir
- Stopwatch or timer
- Sterile pipettes
- CO_2 Incubator
- Liquid nitrogen
- Heated stage

Selection of appropriate vitrification device

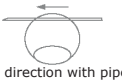
Use a legally marketed device indicated for use in blastocyst vitrification procedures. Use a closed system to prevent the potential risk of viral contamination using open systems where the sample comes in direct contact with liquid nitrogen. The device needs to meet the following rate of cooling: minimum 1.800ºC/min (High security straw)

Directions for Use

Vitrifying Blastocysts with VitriBlast™

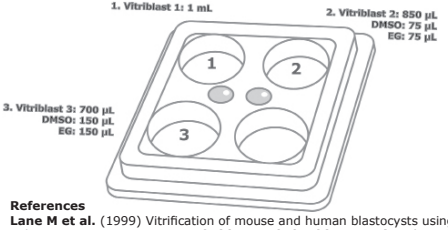
Work on a heated stage at all times when manipulating the blastocyst. Do not let the blastocyst remain exposed to the microscope light during incubations.

1. Label the 4 well culture dish with the patient ID and each well with each solution number
2. 1 hr prior to use or the day before; remove the DMSO from the refrigerator, and let liquify in RT
3. Prepare a 4 well culture dish by adding 1 mL of VitriBlast 1 to the first well
4. Add 850µL of VitriBlast™ 2, 75µL of DMSO and 75µL EG respectively to the second well. Mix thoroughly
5. Add 700µL of VitriBlast™ 3, 150µL of DMSO and 150µL EG respectively to the third well. Mix thoroughly
6. Incubate at 37°C in 5-6% CO_2 for **30 minutes**
7. This step is optional for blastocysts with smaller blastocoelic cavities but recommended for all other blastocysts. During the 30 minute incubation, collapse the blastocyst by pipette tip under microscope. Punctuate as far from ICM as possible
8. Transfer the punctured and collapsed blastocyst to VitriBlast™ 1. Incubate **1.5-2 minutes** on the heated stage



Puncture direction with pipette

9. Transfer the blastocyst to VitriBlast™ 2 by aspirating VitriBlast2 into the pipette tip, collect the blastocyst and transfer to VitriBlast™ 2 (well 2). Incubate for exactly 2 minutes on the heated stage
10. During the 2 minute incubation; prepare 2 x 10µL drops of VitriBlast™ 3 in the middle of the dish (see diagram). At the correct time, move the blastocyst by aspirating VitriBlast™ 3 from the well into the pipette tip, collect the blastocyst from solution 2 in the second well, and transfer it to solution no 3 in the 10µL droplet
11. Load blastocyst onto vitrification device in the smallest volume of VitriBlast™ 3 possible
12. The blastocyst must remain in VitriBlast™ 3 for **30-45 seconds**, including the time on the device
13. Plunge quickly into liquid nitrogen
14. Transfer to storage in liquid nitrogen. Do not let the blastocyst come in contact with room tempered air during transfer



Temperature limit

Use by - see label

Sterilized Using Aseptic Processing Techniques

Batch code

Consult instructions for use

Manufacturer

References

Lane M et al. (1999) Vitrification of mouse and human blastocysts using a novel cryoloop container-less technique. Fertility and Sterility. Vol 72, No 6, pp1073-1078, **Mukaida T, Takahashi K, Kasai M.** (2003) Blastocyst cryopreservation: ultrarapid Vitrification using cryoloop technique. Reproductive BioMedicine Online., Vol. 6, No. 2, pp221-225, **Mukaida T et al.** (2003) Vitrification of human blastocysts using cryoloops: clinical outcome of 223 cycles. Hum Reprod., Vol. 18, No. 2, pp384-391, **Hardarson T et al.** (2006) Vitrification and warming human blastocysts by use of a laser to artificially induce blastocyst collapse prior to vitrification. Acta Obstet Gynecol Scand., 86 p. 119-120, **Kartberg A-J et al**, (2008), Vitrification with DMSO protects embryo membrane intergrity better than solution without DMSO, Reproductivie Medicine Online, Volume 17, 3 September 2008. ***For more references please visit nidacon.com***