Vit Kit - Freeze NX (Vitrification Kit for Freezing)

with Gentamicin and DSS

For distribution in Japan only.

Catalog # 90188-WK

- **Equilibration NX - ES (white cap)** **9 x 1 mL**
- **Vitrification NX - VS (blue cap)** **9 x 1 mL**

For assisted reproductive procedures.

Glossary of Symbols*:

Catalog Number

Lot Number

Sterilized using aseptic processing techniques (filtration)


Expiration: Year - Month - Day


Caution, consult accompanying documents


Consult instructions for use

Storage Temperature 2-8°C

Do not resterilize


Do not re-use


Do not use if package is damaged

Phthalate, DBP, DEHP


Manufacturer

*Symbol Reference - **EN ISO 15223-1,** Medical devices – Symbols to be used with medical device labels, labeling.

FUJIFILM Irvine Scientific, Inc.
2511 Daimler Street, Santa Ana, California 92705 USA
Telephone: 1 949 261 7800 • 1 800 437 5706 • Fax: 1 949 261 6522 • www.irvinesci.com

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ENGLISH

INDICATIONS FOR USE
Vit Kit - Freeze NX is intended for use in assisted reproductive procedures for vitrification and storage of human oocytes (MII), pronuclear (PN) zygotes through day 3 cleavage stage embryos and blastocyst stage embryos.

DEVICE DESCRIPTION:

Equilibration NX - ES is a dual buffered solution (HEPES & MOPS) of Continuous Single Culture medium (CSCM) containing Gentamicin Sulfate, 7.5% (v/v) of each DMSO and ethylene glycol, and 20% (v/v) Dextran Serum Supplement (DSS).

Vitrification NX - VS is a dual buffered solution (HEPES & MOPS) of CSCM containing Gentamicin Sulfate, 15% (v/v) of each DMSO and ethylene glycol, 20% (v/v) DSS and 0.5 M Trehalose.

DSS is a protein supplement consisting of 50 mg/mL therapeutic grade Human Serum Albumin (HSA) and 20 mg/mL Dextran. DSS is used at 20% (v/v) in Vit Kit – Freeze NX for a final concentration of 10 mg/mL HSA and 4 mg/mL Dextran.

These solutions are to be used in sequence according to the step-wise microdrop vitrification protocol.

COMPOSITION

Salts & Ions
Potassium Phosphate
Sodium Chloride
Potassium Chloride
Magnesium Sulfate
Calcium Chloride

Buffer
Sodium Bicarbonate
HEPES
MOPS

Amino Acids
L-Arginine
Glycine
L-Histidine
L-Lysine
L-Proline
L-Tyrosine
L-Alanine
L-Aspartic Acid
L-Asparagine
L-Glutamic Acid
L-Isoleucine
L-Leucine
L-Alanyl-L-Glutamine
L-Methionine
L-Phenylalanine
L-Serine
L-Threonine
L-Tryptophan
L-Valine
L-Cystine

Antioxidants
Sodium Citrate
EDTA

Antibiotics
Gentamicin Sulfate

Energy Substrates
Dextrose
Sodium Pyruvate
Sodium Lactate

Protein
Human Serum Albumin, HSA

Cryoprotectants
Dextran
Trehalose
Ethylene Glycol
Dimethylsulfoxide

QUALITY ASSURANCE
The solutions in Vit Kit - Freeze NX are membrane filtered and aseptically processed according to manufacturing procedures which have been validated.

Each lot of Vit Kit - Freeze NX receives the following tests:
- Endotoxin by Limulus Amebocyte Lysate (LAL) methodology (≤0.6 EU/mL) by USP Bacterial Endotoxins <85> and Ph. Eur. 2.6.14
- Mouse Embryo Assay (one-cell) (≥ 80% expanded blastocyst)
- Sterility by the current USP Sterility Test <71>, Ph. Eur. 3.2 (Pass)

All results are reported on a lot specific Certificate of Analysis which is available upon request.

MATERIALS REQUIRED BUT NOT INCLUDED
- Vitrification device of choice
- Sterile petri dishes (50 X 9 mm, Falcon 351006 or equivalent)
- Cryotubes (4.5 mL) or goblets and cryocanes
- Hyaluronidase (Catalog #90101) for oocyte vitrification
- Disposable gloves
- Transfer pipettes (pulled glass pipettes or micropipette tips with an inner tip diameter of ~200µm)
- Tweezers or forceps
- Stopwatch or timer
- Liquid nitrogen reservoir (dewar or styrofoam container with lid, 1-2 L volume)
- Liquid nitrogen (sufficient volume to achieve 4 inch depth in reservoir)

DIRECTIONS FOR USE
Vit Kit - Freeze NX component requirements (per application):
- Equilibration NX – ES (ES) 50 µL
- Vitrification NX – VS (VS) 50 µL

VITRIFICATION PROTOCOL:
NOTE: Procedures are to be done at room temperature (20-27ºC). DO NOT use heated microscope stage for the following procedures. CAUTION: Minimize exposure of specimen to light during equilibration in ES and VS solutions.

1. Bring the quantity to be used of ES, and VS to room temperature (20-27°C).
   NOTE: Avoid bringing the entire vials of ES, and VS to room temperature repeatedly when a partial of the solution is needed each time. It is better to aliquot the quantity to be used and return the vials to 2-8°C right after aliquoting.
2. Fill the liquid nitrogen reservoir with liquid nitrogen (LN_2) – sufficient to achieve a depth of 4 inches or to completely submerge cryotube on cane – and place close to microscope. Attach a cryotube or goblet (uncapped) to the bottom clamp of a cryocane and submerge in the liquid nitrogen in preparation for storage of the vitrified specimens.
3. Determine the number of specimens to be vitrified.
4. Label each sterile petri dish (or lid) and Cryo storage device with necessary information.
5. Carefully examine the vitrification device before starting procedure
6. Gently invert each vial of ES and VS to mix contents before use.

EMBRYO (PN to Blastocyst) Vitrification Protocol:

1. Aseptically dispense one- 50 µL drop of ES on an inverted lid of Petri dish (Figure 1).
2. Remove the culture dish with embryo(s) from the incubator and check the quality of the specimen(s) under microscope. Where possible, select only the best quality embryo(s) for vitrification.
3. Carefully transfer the specimen (up to two at one time) with a minimal volume of medium from the culture dish to the drop of ES and start the timer.
   Embryos should equilibrate in the ES drop slowly by free-fall for 6-10 minutes.
   NOTE 1: The specimen will shrink and then gradually return to its original size, which indicates that the equilibration is complete.
   CAUTION: Minimize the exposure of specimen(s) to light during equilibration in ES and VS drops.
4. During this equilibration time in ES, aseptically dispense one-50 µL drop of VS solution as shown in Figure 4 and prepare vitrification device of choice for loading.
5. Rinse and fill the transfer pipet tip with VS immediately before equilibration in ES is complete, and draw up the specimen(s) with minimal volume of ES into the pipet tip and transfer into the drop of VS for a minimum of 30 seconds. Unload embryos to the bottom of VS. While unloading, embryos will float to the top of VS. To ensure complete rinse with VS, gently move the embryos back to the bottom center of VS by pipetting.
   NOTE 2: During this process, embryos will be dehydrated and float back again.
6. Load and seal the Vitrification device as directed by manufacturer
7. Place the vitrified Vitrification device of choice into the submerged LN_2 filled cryotube or goblet (on the cryocane) Figure 3. Cap the cryotube (or goblet) or attach up-side-down with another uncapped cryotube in order to secure the vitrified device in liquid nitrogen.
8. Move the LN_2 reservoir close to the LN_2 cryo-freezer and transfer the cryocane with contents to the cryo-freezer for long-term storage.

For additional details on the use of these products, each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

STORAGE INSTRUCTIONS AND STABILITY
Store the unopened vials refrigerated at 2°C to 8°C. When stored as directed, Vit Kit – Freeze NX Solutions are stable until the expiration date shown on the vial labels.

As human source material is present in the product it may develop some particulate matter during storage. This type of particulate matter is not known to have an effect on product performance.

PRECAUTIONS AND WARNINGS
This device is intended to be used by staff trained in assisted reproductive procedures. These procedures include the intended application for which this device is intended.

The user facility of this device is responsible for maintaining traceability of the product and must comply with national regulations regarding traceability, where applicable.

Do not use any vial of solution which shows evidence of damage, leaking, particulate matter, cloudiness. Discard the product in accordance with applicable regulations.

To avoid problems with contamination, handle using aseptic techniques.

Currently, research literature indicates the long-term effects of vitrification on oocytes and embryos remains unknown.

Do not use any bottle in which the sterile packaging has been compromised.

EU: 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 Pharmacopeia specifications by established processes. It is strongly recommended that every time FUJIFILM Irvine Scientific, Inc. Reproductive Media Products culture media are administered to 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.

US: This product contains Human Serum Albumin (HSA). Human source material used in the manufacture of this product has been tested by FDA-licensed kits and found to be non-reactive to the antibodies to Hepatitis C (HCV), and antibodies to Human Immunodeficiency Virus (HIV). However, no test method offers complete assurance that products derived from human sources are noninfectious. Handle all human source material as if it were capable of transmitting infection, using universal pre-cautions. Donors of the source material have also been screened for CJD.

CONTRAINDICATION
Product contains Gentamicin Sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.

Figure 1

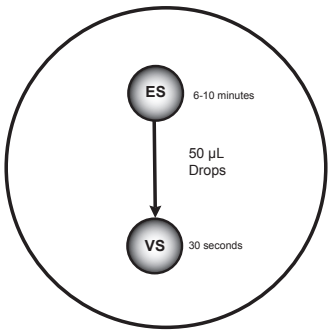

Figure 2