














Vit Kit - Freeze NX (Vitrification Kit for Freezing) with Gentamicin and DSS

Catalog No. 90188 Includes:

- Equilibration NX - ES (white cap)
- Vitrification NX - VS (blue cap)
- Washing NX - WS (red cap)

For assisted reproductive procedures.

Glossary of Symbols*:

| | |
|---|---|
|  | Catalog Number |
|  | Lot Number |
|  | Sterilized using aseptic processing techniques (aseptically filtered) |
|  | Expiration: Year - Month - Day |
|  | Caution, consult accompanying documents |
|  | Consult instructions for use |
|  | Storage Temperature 2-8°C |
|  | Do not re-use |
|  | Do Not Re-Sterilize |
|  | Do Not Use If Package Is Damaged |
|  | Phthalate, DBP, DEHP 1mL container |
|  | Manufacture |
|  | U.S. Caution: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner. |

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


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Figure 1:

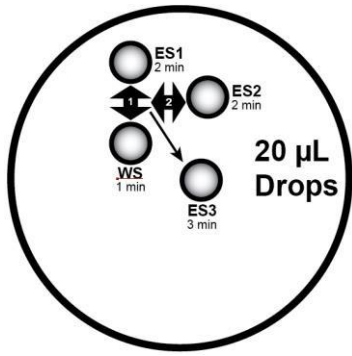
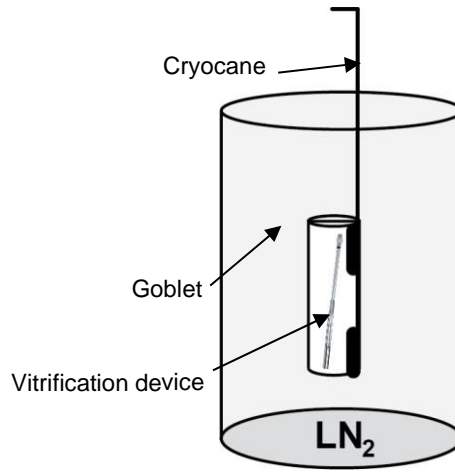


Figure 3



Oocyte Vitrification After Step 7

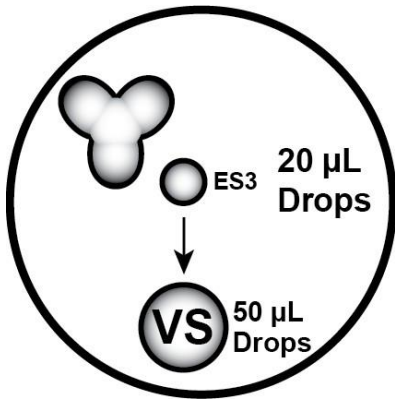


Figure 4:

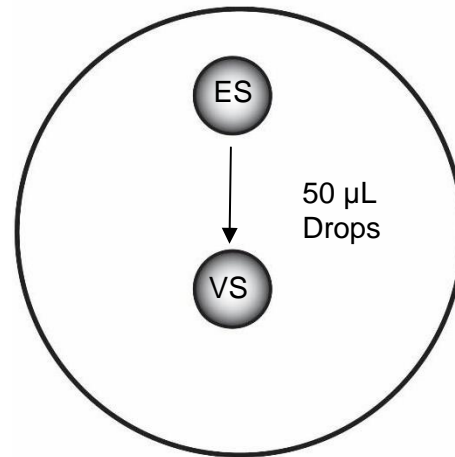
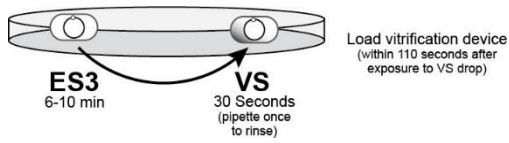


Figure 2:



ENGLISH

U.S. CAUTION: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.

INDICATION 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 (ES) is a HEPES/MOPS buffered solution 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 (VS) is a HEPES/MOPS buffered solution of Continuous Single Culture medium (CSCM) containing Gentamicin Sulfate, 15% (v/v) of each DMSO and ethylene glycol, 20% (v/v) DSS and 0.5 M Trehalose.

Washing NX- WS (WS) is a dual buffered solution (HEPES and MOPS) of CSCM containing Gentamicin Sulfate and 20% DSS.

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

Antioxidant

Sodium Citrate
ETDA

Antibiotics

Gentamicin Sulfate

Buffer

Sodium Bicarbonate
HEPES
MOPS

Energy Substrates

Dextrose
Pyruvic Acid
Sodium Lactate

Amino Acids

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

Protein

Human Serum Albumin

Cryoprotectant

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)
- Mouse Embryo Assay (one-cell) ($\geq 80\%$ expanded blastocyst)
- Sterility by the current USP Sterility Test <71> (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)
- Disposable gloves
- Transfer pipettes (pulled glass pipettes or micropipette tips with an inner tip diameter of $\sim 200\mu\text{m}$)
- 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):
60 μL for **Oocyte Vitrification Protocol**
Or
50 μL for **Embryo Vitrification Protocol**
- Vitrification NX – VS (VS):
50 μL for either Vitrification Protocol
- Washing NX – WS (WS):
20 μL for **Oocyte Vitrification Protocol**

VITRIFICATION PROTOCOL:

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

- Bring the quantity to be used of ES, VS, and WS to room temperature (20-27°C). NOTE: Avoid bringing the entire vials of ES, VS and WS 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. Washing NX (WS) is used for oocyte vitrification.
- 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.
- Determine the number of specimens to be vitrified.
- Label each sterile petri dish (or lid) and vitrification device with necessary information.
- Gently invert each vial of ES and VS to mix contents before use.
- Prepare dish with droplets of solutions for Vitrification Procedure as follows:

A. OOCYTE (MII) Vitrification Protocol:

NOTE 1: Retrieved oocytes are denuded with Hyaluronidase to confirm they are MII.

NOTE 2: Refer to Section B for embryo vitrification protocol.

- Aseptically dispense 20 μL drop of WS, ES1 & ES2 in close proximity and ES3 on an inverted lid of sterile petri dish as shown in **Figure 1**, and place the dish on the microscope stage:
 - one- 20 μL drop of WS
 - three- 20 μL drops (60 μL total) of ES (ES1, ES2, ES3)
- Remove the culture dish containing MII oocytes from the incubator and check the quality of the specimens under microscope. Where possible, select only the best quality MII stage oocyte(s).
CAUTION: Minimize the exposure of the specimen(s) to light during equilibration in the WS, ES and VS drops.
- Transfer the oocyte (up to 2 at a time) with minimal volume of medium from the culture dish (in incubator) into the 20 μL drop of WS for one minute.

4. Merge the drop of WS to ES1 (See **Figure 1**, arrow 1) with the tip of the transfer pipette and allow spontaneous mixing of the two solutions to occur for 2 minutes.
5. Then merge the drop of ES2 (arrow 2) to the previously merged drops and leave for 2 minutes.
6. Transfer the oocyte(s) with minimal volume of solution from merged drop to ES3 drop for 6-10 minutes. Note: equilibration of oocyte(s) in ES3 is complete when the thickness of the zona pellucida and perivitelline space is equal. The oocyte(s) will settle to the bottom of the drop within 3 minutes.
7. During the equilibration time in ES3, aseptically dispense one (1) 50 μ L drop of VS prior to complete equilibration and prepare the Vitrification device of choice for loading (**Figure 2**).
NOTE: Carefully examine the vitrification device and tip before starting procedure
8. The following steps (9-13) should be completed in 80-110 seconds. **CAUTION:** Exposure of specimen(s) to VS should be limited to prevent cytotoxicity. Specimen(s) tend to float in VS, so adjust the focus through the microscope to maintain continuous visualization during exposure and keep the tip of the transfer pipette nearby to assure rapid transfer between drops. Refer to **Figure 2**.
9. 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 30-60 seconds**.
 - a. Unload oocytes to the bottom of VS. While unloading, oocytes will float to the top of VS. This will take about 15 – 30 seconds. To ensure complete rinse with VS, gently move the oocytes back to the bottom center of VS by pipetting.
 - b. During this process, oocytes will be dehydrated and float back again, taking another 15 – 30 seconds.
- 10. Load and seal the Vitrification device as directed by manufacturer.**
11. Place the vitrified specimen(s) on the Vitrification device of choice into the submerged LN₂ 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.
12. Move the LN₂ reservoir close to the LN₂ cryo-freezer and transfer the cryocane with contents to the cryo-freezer for long-term storage.

B. EMBRYOS (PN to Blastocyst) Vitrification Protocol:

1. Aseptically dispense one- 50 μ L drop of ES on an inverted lid of Petri dish (Figure 4).
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: 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 μ L 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 30 seconds**.
 - a. Unload embryos to the bottom of VS. While unloading, embryos will float to the top of VS. This will take about 15 seconds. To ensure complete rinse with VS, gently move the embryos back to the bottom center of VS by pipetting.
 - b. During this process, embryos will be dehydrated and float back again, taking another 15 seconds.
- 6. Load and seal the Vitrification device as directed by manufacturer**
7. Place the vitrified Vitrification device of choice into the submerged LN₂ 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 LN₂ reservoir close to the LN₂ 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.

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.