Vit Kit® - Freeze
Vitrification Freeze Kit for Embryos (PN through Blastocyst Stage)

Catalog No. 90133DSO Includes:
• Equilibration Solution - ES (white cap) 2 x 1 mL Vials
• Vitrification Solution - VS (blue cap) 2 x 1 mL Vials

For assisted reproductive procedures.
Caution: Federal law restricts this device to sale by or on the order of a physician or a practitioner trained in its use.

Caution: The user should read and understand the Directions for Use, Warnings and Precautions, and be trained in the correct procedure before using the Irvine Kits for vitrification of human blastocysts.

INTENDED USE

Vit Kit® - Freeze is intended for use in the vitrification of human embryos from pronuclear (PN) zygotes through blastocyst stage. This kit can be used with Irvine Scientific's CryoTip® (Catalog No. 40709), and Connector (Catalog No. 40736) or other cleared storage device. This kit is designed for use with Irvine Scientific's Vit Kit® - Thaw (Catalog No. 90137DSO) for optimal recovery of specimens.

PRECAUTIONS AND WARNINGS

This device is intended to be used by staff trained in assisted reproductive procedures that include the indicated application for which the device is intended.

As an added precaution during the preparation procedure, if using a CryoTip we recommend that each CryoTip be carefully examined when taken out of the package. Prior to use, the CryoTips should be examined under a suitable magnification (40x power) for possible damage (such as tip breakages or cracks) that may have occurred during transport.

Do not use any vial of solution which shows evidence of damage, leaking, particulate matter, cloudiness or not red to reddish-orange in color. Discard the product in accordance with applicable regulations.

To avoid problems with contamination, handle using aseptic techniques.

Vitrification Freeze Kit Solutions contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitized to this antibiotic.

The long term safety of vitrification on children born following this method of embryo cryopreservation is unknown.

*Human source materials used in the manufacture of this product have been tested by FDA licensed kits, and found to be non-reactive to the antibodies for Hepatitis B surface antigen (HbsAg), antibodies to Hepatitis C (HCV) and antibodies to Human Immunodeficiency Virus, (HIV). Donors of the source material have also been screened for CJD. However, no test method offers complete assurance that products derived from human sources are noninfectious. Handle all human source material as if it is capable of transmitting infection, using universal precautions.
PRODUCT DESCRIPTION

**Equilibration Solution-ES** is a HEPES buffered solution of Medium-199 containing gentamicin sulfate (35 µg/mL), 7.5% (v/v) of each DMSO and ethylene glycol and 20% (v/v) Dextran Serum Supplement*.

**Vitrification Solution-VS** is a HEPES buffered solution of Medium-199 containing gentamicin sulfate (35 µg/mL), 15% (v/v) of each DMSO and ethylene glycol, 20% (v/v) Dextran Serum Supplement* and 0.5 M sucrose.

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

QUALITY ASSURANCE

The solutions in Vit Kit-Freeze are membrane filtered and aseptically processed according to manufacturing procedures which have been validated to meet a sterility assurance level (SAL) of $10^{-3}$.

Each lot of Vit Kit-Freeze receives the following tests:

**Solutions:**
- Endotoxin by LAL methodology
- Biocompatibility by mouse embryo assay (one-cell)
- Sterility by the current USP Sterility Test <71>
- Albumin Test

**CryoTips:**
- Endotoxin by LAL methodology
- Biocompatibility by mouse embryo assay (one-cell)
- Sterility by the current USP Sterility Test <71>

**Connectors:**
- Sterility by the current USP Sterility Test <71>

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

MATERIALS REQUIRED BUT NOT INCLUDED

- Irvine Scientific CryoTip (Catalog No. 40709)
- Irvine Scientific Connector (Catalog No. 40736)
- Closed Storage Device (Refer to Cryostorage Device Requirements below)
- Sterile Petri Dishes (50 X 9 mm, Falcon 351006 or equivalent)
- Cryotubes (4.5 mL) or goblets and cryocanes
- Disposable gloves
- Hamilton GASTIGHT® Syringe (50 µL) catalog #80901
- Transfer pipettes (pulled glass pipettes or micropipette tips with an inner tip diameter of ~200 µm)
- Tweezers
- Impulse Heat Sealer
- 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)
CRYOSTORAGE DEVICE REQUIREMENTS

- Closed Cryostorage Device
- Use a legally marketed storage device indicated for use in pronuclear through blastocyst stage embryo vitrification procedures.
- Use a closed storage system to prevent the potential risk of viral or other contamination of samples.
- Use a closed storage system taking into consideration cooling and warming rates of 12,000°C/minutes and 24,000°C/minute respectively.

DIRECTIONS FOR USE

Vit Kit - Freeze component requirements (per application) for embryo vitrification:
- Equilibration Solution (ES): 50 µL
- Vitrification Solution (VS): 50 µL
- Cryostorage device
  For CryoTips:
  - 1 CryoTip (stores up to two specimens)
  - 1 Connector

Vitrification Protocol for PN zygotes through blastocyst stage embryos:

NOTE: Procedures are to be performed at room temperature (20-27º 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.

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 (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 cryostorage device with necessary information.

5. Gently invert each vial of ES and VS twice to mix contents before use.

6. Aseptically dispense one- 50 µL drop of ES on an inverted lid of Petri dish.

7. 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.
8. 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.

9. **During this equilibration time in ES:**
   - aseptically dispense (1) 50 µL drop of VS 2 minutes prior to complete equilibration and
   - prepare the cryostorage device for loading according to manufacturer's instructions.

For CryoTip:
Prepare the CryoTip for loading by inserting the wide end of the CryoTip into the narrow end of the connector until aligned with **Mark #4** on the CryoTip to form a tight seal. Securely attach the Hamilton syringe to the wide end of the Connector. Assure a tight seal with the connector to prevent aspiration into the CryoTip by capillary action. Keep the metal cover sleeve over the fine pulled tip to protect it until ready to load specimens.

**Figure 1:**

![Diagram](image)

50 µL Drops

ES 6-10 min

VS 30 Seconds (pipette once to rinse)

Load, seal and plunge CryoTip within 110 sec after initial exposure to VS

10. The following steps (11-16) should be completed in **90-110 seconds.**

**CAUTION:** Exposure of specimens 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 VS drops. Refer to Figure 1.

11. After equilibration in ES is complete, draw up some ES into the transfer pipet and transfer the specimen(s) with minimal volume from the drop of ES into the drop of VS for **30 seconds.**

12. Load specimen(s) into the closed cryostorage device according to the manufacturer's instructions, and plunge the cryostorage device directly into liquid nitrogen (LN₂).
a. For CryoTips: Load and heat seal the CryoTip as follows (See Figure 2):

- Slide the metal cover sleeve up along the CryoTip to expose the fine fragile tip end.
- Handling the CryoTip and Hamilton syringe while observing under the microscope, carefully aspirate a small volume of VS to the **Mark #1** on the CryoTip.
- Continue observation under the microscope and gently aspirate the specimen with VS to the **Mark #2** on the CryoTip.
- Now observe the CryoTip directly and aspirate more VS to the **Mark #3**.
- Specimen must be located between **Mark #2** and **Mark #3**.
- Heat seal (**Seal #1**) the CryoTip on (or just below) **Mark #1**, and slide the cover sleeve back down to cover and protect the fine fragile tip.
- Carefully remove the connector and Hamilton syringe and then heat seal (**Seal #2**) at the thick end of the CryoTip **above** the **Mark #4**.

b. Plunge the covered CryoTip directly into liquid nitrogen (cooling at a rate of \(-12,000^\circ\) C/min).

c. Place the vitrified CryoTip into the submerged LN\(_2\) filled cryotube or goblet (on the cryocane). Cap the cryotube (or goblet) or attach up-side-down with another uncapped cryotube in order to secure the vitrified CryoTip in liquid nitrogen. Each cryotube can hold up to 4 CryoTips.

13. Place the vitrified cryostorage device in appropriate container filled with LN\(_2\) (or LN\(_2\) vapor) as indicated by manufacturer's instructions.

14. Move the LN\(_2\) reservoir close to the LN\(_2\) cryo-freezer and transfer the cryocane or container with vitrified 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, Vitrification Freeze Kit Solutions are stable until the expiration date shown on the vial labels.

Do not use media for more than eight (8) weeks once containers have been opened.

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.

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REFERENCES


Symbols:

- **REF**: Catalog Number
- **LOT**: Lot Number
- **STERILE**: Sterilized using aseptic processing techniques (filtration)
- **Expiration**: Year - Month - Day
- **Caution**: See instructions for use
- **Storage Temperature**: 2°C - 8°C
- **Manufacturer**