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

Catalog No. 90137DSO Includes:
• Thawing Solution - TS (yellow cap) 4 x 2 mL Vials
• Dilution Solution - DS (orange cap) 1 x 2 mL Vials
• Washing Solution - WS (red cap) 1 x 2 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 embryos.

**INTENDED USE**

Vit Kit® - Thaw is intended for use in the warming of pronuclear (PN) zygotes through blastocyst stage embryos, which were vitrified using Irvine Scientific’s Vitrification Freeze Kit (Vit Kit® - Freeze, Catalog No. 90133DSO) for embryos.

**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.

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 Thaw 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 embryo 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**

**Thawing Solution-TS** is a HEPES buffered solution of Medium-199 containing gentamicin sulfate (35 µg/mL), 1.0 M sucrose and 20% (v/v) Dextran Serum Supplement*.

**Dilution Solution-DS** is a HEPES buffered solution of Medium-199 containing gentamicin sulfate (35 µg/mL), 0.5M sucrose and 20% (v/v) Dextran Serum Supplement*.
Washing Solution-WS is a HEPES buffered solution of Medium-199 containing gentamicin sulfate (35 µg/mL) and 20% (v/v) Dextran Serum Supplement*.

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

QUALITY ASSURANCE
The solutions in Vit Kit-Thaw 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-Thaw 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

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

FOR WARMING CRYOTIP AS THE STORAGE DEVICE: MATERIALS REQUIRED BUT NOT INCLUDED
- Irvine Scientific Connector (Catalog No. 40736) or adaptor
- Sterile Petri Dishes (50 X 9 mm, Falcon 351006 or equivalent)
- 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
- 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)
- Sharp scissors (sterile)
- 37°C waterbath (>500 mL)
- Culture Medium (with 20% protein) appropriate for developmental stage of specimen to be recovered.
- Prepare and pre-equilibrate dish with culture medium to 37°C in CO$_2$ incubator prior to thawing specimens.

DIRECTIONS FOR USE
Vit Kit-Thaw components (per application):
- 50 µL of Thawing Solution-TS
- 50 µL of Dilution Solution-DS
- 100 µL of Washing Solution-WS
- 1 Connector

WARMING PROTOCOL
(FOR EMBRYOS)
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 manipulations through warming solutions.

1. Bring the quantity to be used of TS, DS and WS to room temperature (20-27°C) prior to warming vitrified specimens.

   NOTE: Avoid bringing the entire vials of TS, DS and WS to room temperature repeatedly when a small quantity 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 (~80 % full) and place close to the LN$_2$ freezer containing the specimens to be warmed.

3. Remove the canes with goblets containing the CryoTips with vitrified specimens from liquid nitrogen storage and transfer them into the liquid nitrogen filled reservoir.

   Place the reservoir close to the microscope for rapid manipulation.

   CAUTION: Make sure that CryoTips remain submerged in LN$_2$ (in goblet) during transfer from storage to LN$_2$ reservoir to prevent uncontrolled thawing of specimens.

4. Label a sterile petri dish (or lid) with necessary information.

5. Gently invert each vial of TS, DS and WS twice to mix contents before use.

6. To set up warming dish, aseptically dispense:
   - One (1) 50 µL drop of TS
   - One (1) 50 µL drop of DS
   - Two drops of WS will be set up later at Step 15

   Figure 1.

7. Place the 37°C waterbath close to the microscope. Have the following nearby: a transfer pipet and tips, sterile sharp scissors, Hamilton syringe and sterile wipes.
8. Using tweezers (or tongs), quickly remove CryoTip from LN\textsubscript{2} and within 1 second fully immerse the device in the 37°C waterbath (>500 mL) and swirl device gently for 3 seconds. Swirling the device is critical to ensure the most rapid warming rate (+24,000°C/min).

**Figure 2.**

![Diagram](image)

9. Remove CryoTip from the waterbath and promptly remove metal cover sleeve from device by firmly grasping the lower end of the cover sleeve and pulling away from the CryoTip. Gently wipe away any water with a sterile dry tissue ensuring the tip of the device is dry.

10. Using sterile medical grade sharp scissors make Cut #1 below seal at wide end of CryoTip.

11. Withdraw the plunger of the syringe (with connector attached) to the half way position. Gently attach CryoTip to connector and syringe (or pipette).

12. Place fine tip end over the prepared warming dish and quickly make Cut #2 above the seal at the fine end.

13. While visualizing under the microscope, dispense contents of CryoTip as a small drop directly adjacent to TS drop. Once you visualize the specimen(s) touch the CryoTip contents drop to TS drop with end of CryoTip to mix. Set timer for 1 minute and leave undisturbed.

**NOTE: AVOID BUBBLES WHILE DISPENSING THE CONTENTS.**

**NOTE:** The specimens will shrink and float to the top of the drop.

**NOTE:** After each transfer of the specimen(s), blow out any remaining fluid in the transfer pipet and draw up some solution from the next drop prior to the next manipulation. Avoid creating bubbles during the transfers.

14. Transfer specimen(s) to DS, for 4 minutes. Gently pipette specimens once to ensure complete rinse in DS.

**NOTE:** The specimen will remain shrunken during exposure to DS.

15. During the 4 minute exposure in DS aseptically dispense two (2) 50 μL drops of WS (WS1, WS2) as in **Figure 1**.

16. Transfer specimen(s) to WS1 then WS2 for 4 minutes each undisturbed.

**NOTE:** The specimen(s) should re-expand to the original size within 2-3 minutes in WS.

17. There are two options for warmed EMBRYO(S):
   a) For immediate transfer to patient: transfer EMBRYO(S) to pre-equilibrated ‘transfer’ medium containing 20% (v/v) protein supplement or 12 mg/mL.
   b) For further culture: transfer EMBRYO(S) to pre-equilibrated culture medium containing 20% (v/v) protein supplement or 12 mg/mL for a 4 hour recovery period. After recovery period transfer EMBRYO(S) to culture medium with 10% (v/v) protein and incubate accordingly until desired developmental stage has been reached for transfer to patient.
FOR WARMING OTHER CRYOSTORAGE DEVICES:
MATERIAL REQUIRED BUT NOT INCLUDED

- Sterile 4-well dish (Nunc 179830, 144444 or equivalent), or organ culture dish (BD Falcon 353037)
- Disposable gloves
- Transfer pipettes
- Tweezers
- Stopwatch or timer
- Liquid nitrogen reservoir
- Liquid Nitrogen
- Scissors
- Culture Medium with 20% protein, pre-equilibrated to 37°C in CO₂ incubator prior to thawing procedure.
- 37°C incubator without CO₂ or heating stage

DIRECTIONS FOR USE
Vit Kit – Thaw components (per application)
• 250 µl of Thawing Solution-TS
• 50µL of Dilution Solution-DS
• 50 µL of Washing Solution-WS

WARMING PROTOCOL
(FOR EMBRYOS)
NOTE: The warming steps include plunging the device into the 37°C TS and subsequent diluting and washing in DS and WS at room temperature

1. Set up warming dishes (see Figure 4):
   • At 37°C: Aseptically dispense a minimum of 250 µl of TS into a sterile 4-well dish or an organ culture dish and place it in a 37°C incubator without CO₂ or on a heating stage at least 30 minutes prior to thawing procedure

2. Identify cryostorage device(s) to be thawed from LN₂ storage and quickly transfer to LN₂ filled reservoir in preparation for warming procedure.
3. Place LN₂ reservoir close to microscope for subsequent rapid manipulation.
4. Remove TS dish from 37°C incubator or heating stage and place it under focus on top of the microscope stage.
5. Warm the selected cryostorage device and dispense specimen(s) into 37°C TS solution according to manufacturer’s instructions.
6. Leave specimen(s) in TS for 1 minute.

Steps 7-10 must be performed at room temperature
• At room temperature: Aseptically dispense one (1) 50 µL drop of DS on a sterile Petri dish as in Figure 5.

Figure 5.

7. Transfer specimen(s) to DS for 4 minutes. Gently pipette specimens once to ensure complete rinse in DS.

NOTE: The specimen will remain shrunken during exposure to DS.

8. During the 4 minute exposure in DS, aseptically dispense two (2) 50 µL drops of WS (WS1, WS2) as shown in Figure 5.

9. Transfer specimen(s) to each WS1 then WS2 for 4 minutes each, undisturbed.

NOTE: The specimen(s) should re-expand to the original size within 2-3 minutes in WS.

10. There are two options for warmed EMBRYO(S):
   a) For immediate transfer to patient: transfer EMBRYO(S) to pre-equilibrated ‘transfer’ medium containing 20% (v/v) protein supplement or 12 mg/mL.
   b) For further culture: transfer EMBRYO(S) to pre-equilibrated culture medium containing 20% (v/v) protein supplement or 12 mg/mL for a 4 hour recovery period. After recovery period transfer EMBRYO(S) to culture medium with 10% (v/v) protein and incubate accordingly until desired developmental stage has been reached for transfer to patient.

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 of solutions refrigerated at 2°C to 8°C. When stored as directed, Vitrification Thaw Kit solutions are stable until the expiration date shown on the vial label.

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 A**: Sterilized using aseptic processing techniques (filtration)
- **8°C**: Storage Temperature
- **2°C**: Storage Temperature
- **Caution**: See instructions for use
- **Expiration**: Year - Month - Day
- **Manufacturer**