Innovative Cryo Enterprises L.L.C.

I.C.E. Embryo Vitrification Kit

Vitrification Media V1, V2, V3

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ICE Embryo Vitrification Instructions For Use Testing and Cautions

Innvative Cryo Enterprises LLC
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Intended Use:
To be used for vitrification of human embryos (Day 1 - Day 4).
Not to be used with morulae or blastocysts.

Quality Control Testing:
Sterility (SAL 10^{-3})
pH
Endotoxin Tested ≤ 0.5 EU/ml (USP)
Mouse Embryo assay (MEA)
Note: The results of each batch are listed on a Certificate of Analysis, which is available upon request.

Storage instructions and stability:
Store in original container at 2°C to 8°C protected from light.
The product is packaged in tubes and can be used multiple times.
Keep media at 2°C to 8°C when not in use, do not keep at room temperature for extended periods of time.

Precautions and Warnings:
Do not use product if:
1) Product packaging appears damaged or if the seal is broken.
2) Expiration date has been exceeded.
3) Any solution becomes discolored, cloudy, turbid, or shows any evidence of microbial contamination.
To avoid contamination problems, handle using aseptic techniques.
Keep away from sunlight.
Consult operating instructions.

ICE Embryo vitrification and thaw solutions contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure the patient is not sensitized to this antibiotic.

The long term safety of embryo vitrification on children born using this technique is unknown.

This product contains albumin, a product of human blood.
*Human source materials used in the manufacture of this product have been tested with FDA licensed kits, and found to be non-reactive to the antibodies for Hepatitis B surface antigen (HsbAg), antibodies to Hepatitis C (HCV) and antibodies to Human Immuno-deficiency 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 were capable of transmitting infection, using universal precautions.

Caution: US federal law restricts this device to sale by or on the order of a physician (Rx only) 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 ICE Embryo Vitrification kits for storage of human blastocysts.

Quality Assurance

All solutions 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 ICE Embryo Vitrification & Thaw receives the following tests:
pH
Endotoxin by LAL methodology
Biocompatibility and functionality by mouse embryo assay (MEA)
Sterility by the current USP Sterility Test <71>

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I.C.E. EMBRYO VITRIFICATION PROTOCOL

(for use with 0.25cc or 0.5cc cryo-straws)

PART 1
Materials Required:

- ICE Embryo Vitrification Media V1, V2, & V3

Materials required but not included:

- 0.25cc or 0.5cc sterile cryo-straw(s) or micro-volume device (cryo-top, cryo-tec, cryo-loc, etc.)
- Heat sealer
- Container for liquid nitrogen
- Storage tank
- Sterile petri dishes (60x9mm or 4-6 well dish or similar)
- 1ml sterile syringe
- Stopwatch or Timer
- Liquid nitrogen
- Micropipettes for moving cells (270-300um inner diameter)
- Storage cane and goblet
- Stereomicroscope (Heating plate off)

CAUTION: Use for vitrification of pronuclear or cleavage-stage embryos only. Do not freeze blastocysts.

I.C.E. EMBRYO VITRIFICATION PROTOCOL

PART 2
Preparation for Vitrification:

1) Bring V1, V2, & V3 to room temperature (23-27°C).

2) Label Cryostraw (0.25cc) or storage device with necessary patient information. (1-2 blastocysts per storage device is recommended).

3) Fill container with liquid nitrogen.

4) Turn on heat sealer and test. (If using straws).

5) Label one petri dish or 4-6 well plate: V1, V2, V3 for each straw or device to be stored. (See Figure 2.1).

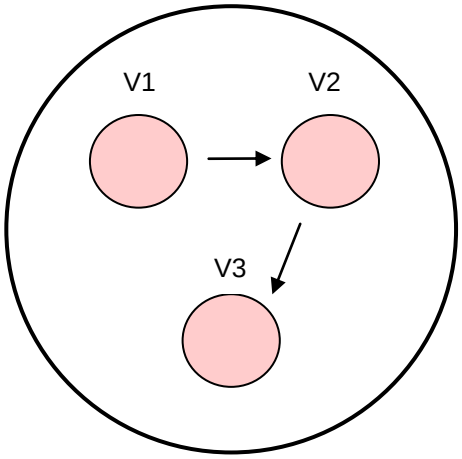


Figure 2.1

I.C.E. EMBRYO VITRIFICATION PROTOCOL

Part 3. Embryo Vitrification

Note: All steps are performed at 23-26°C. Use a timer for all steps.

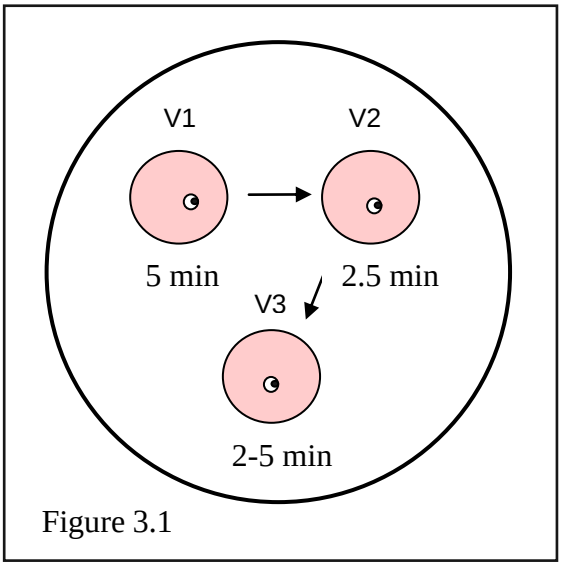


Figure 3.1

1) Pipet 75-100ul of V1, V2, & V3 into the labeled dish and replace the cover. (Do not use oil!)

2) Collect and transfer embryo(s) with minimal media to the V1 drop. *The number of embryos will depend on how many you will vitrify per device.*

3) Incubate in **V1** for **5 min**.

4) Transfer the embryo(s) with minimal media to **V2** for **2.5 min**.

5) Transfer the embryo(s) with minimal media to **V3** for **2-5 min**. Move embryo(s) to a clean area of the drop several times to ensure complete mixing. Figure 3.2. *Be sure to soak the* embryo(s) *at least 2 min in V3 prior to storing in LN2.*

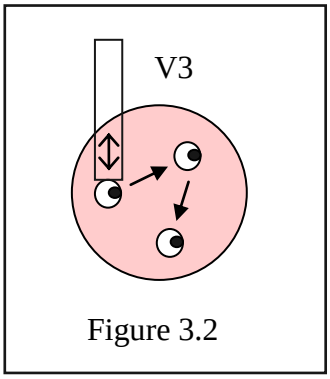
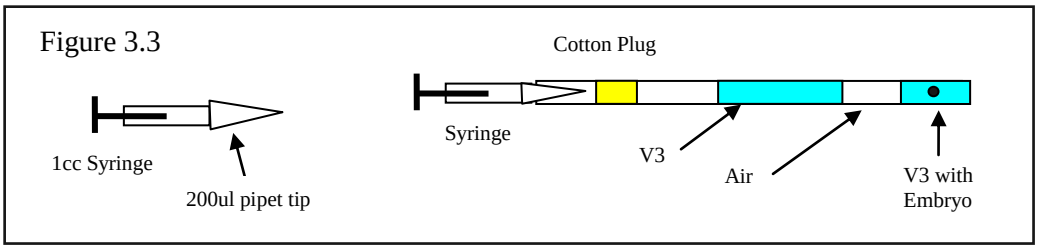


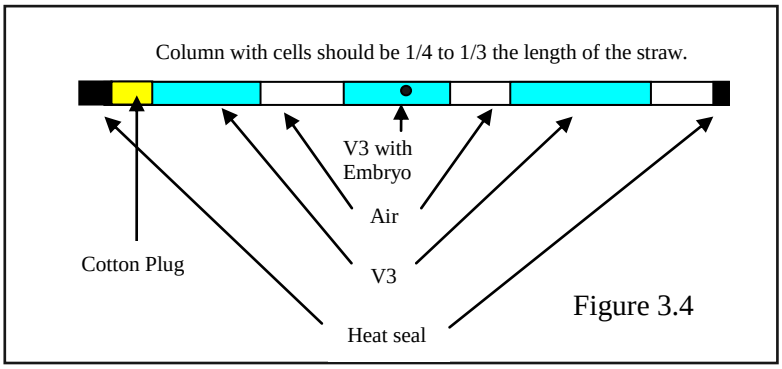
Figure 3.2

Instructions for loading and storing embryo(s) in 0.25cc or 0.5cc straws.

6) Load embryo(s) in a 0.25cc or 0.5cc straw between columns of V3. See Figure 3.3 & 3.4. *Aspirate the first column of V3 to the cotton plug, as this will allow the columns to remain intact.*



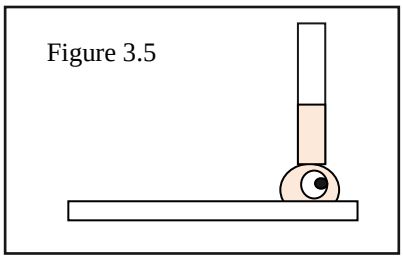
7) Heat seal both ends of the straw. See Figure 3.4. *You must heat-seal both ends of the straw! Periodically check seal under microscope to ensure a good seal.*



8) Plunge and store in LN_2.

Instructions for loading and storing embryo(s) on a micro-volume device.

6) Load embryo(s) onto micro-volume device (cryo-top, cryo-tec, rapid-i, cryo-loc, etc.). Be careful not to load too much media onto device. See Figure 3.5. Always refer to the manufacturers recommendations for loading and storing the device.



7) Plunge and store in LN_2.

For optimal performance:

Push the cotton plug in about 2 cm to have room for heat-sealing.
Label the straw directly or use a flattened 0.5cc straw as a label that can be heat-sealed onto the 0.25cc straw.
Use a 60 mm dish to freeze in. Use large drops (75-100 µl) with no oil overlay.
Use a new dish (new medium) for each set of cells frozen.
Because the time allowed in V3 can be up to 5 min, there is time to prepare multiple straws for vitrification.
Aspirate the first column of V3 to the cotton plug, as this will allow the columns to remain intact.
Store media at 2-8°C.

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I.C.E. Embryo Thaw Kit

Thaw Media T1, T2, T3, T4, T5

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ICE Embryo Thaw Instructions For Use
Testing and Cautions

Innvative Cryo Enterprises LLC
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Linden, New Jersey 07036
USA
973-632-8635

Intended Use:
To be used for thawing embryo(s) frozen using ICE Embryo Vitrification.
Not to be used with morulae or blastocysts.

Quality Control Testing:
Sterility (SAL 10^{-3})
pH
Endotoxin Tested ≤ 0.5 EU/ml (USP)
Mouse Embryo assay (MEA)
Note: The results of each batch are listed on a Certificate of Analysis, which is available upon request.

Storage instructions and stability:
Store in original container at 2°C to 8°C protected from light.
The product is packaged in tubes and can be used multiple times.
Keep media at 2°C to 8°C when not in use, do not keep at room temperature for extended periods of time.

Precautions and Warnings:
Do not use product if:
1) Product packaging appears damaged or if the seal is broken.
2) Expiration date has been exceeded.
3) Any solution becomes discolored, cloudy, turbid, or shows any evidence of microbial contamination.
To avoid contamination problems, handle using aseptic techniques.
Keep away from sunlight.
Consult operating instructions.

ICE Embryo Thaw solutions contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure the patient is not sensitized to this antibiotic.

The long term safety of embryo vitrification on children born using this technique is unknown.

This product contains albumin, a product of human blood.
*Human source materials used in the manufacture of this product have been tested with FDA licensed kits, and found to be non-reactive to the antibodies for Hepatitis B surface antigen (HsbAg), antibodies to Hepatitis C (HCV) and antibodies to Human Immuno-deficiency 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 were capable of transmitting infection, using universal precautions.

Caution: US federal law restricts this device to sale by or on the order of a physician (Rx only) 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 ICE Embryo Thaw kits for recovery of vitrified human pronuclear and cleavage-stage embryos.

Quality Assurance

All solutions 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 ICE Embryo Vitrification & Thaw receives the following tests:
pH
Endotoxin by LAL methodology
Biocompatibility and functionality by mouse embryo assay (MEA)
Sterility by the current USP Sterility Test <71>

I.C.E. EMBRYO THAW PROTOCOL

PART 1
Materials Required:

- ICE Embryo Thaw Media T1, T2, T3, T4, & T5

Materials required but not included:

- Container for liquid nitrogen
- Sterile petri dishes (60x9mm or 4-6 well dish or similar)
- 1ml sterile syringe
- Stopwatch or Timer
- Liquid nitrogen
- Micropipettes for moving cells (270-300um inner diameter)
- Stereomicroscope (Heating plate off)
- Disposable gloves
- Scissors (sterile)
- 30°C water bath
- Mineral oil
- Culture medium appropriate for cleavage stage embryos

I.C.E. EMBRYO THAW PROTOCOL

PART 2
Preparation for Thawing:

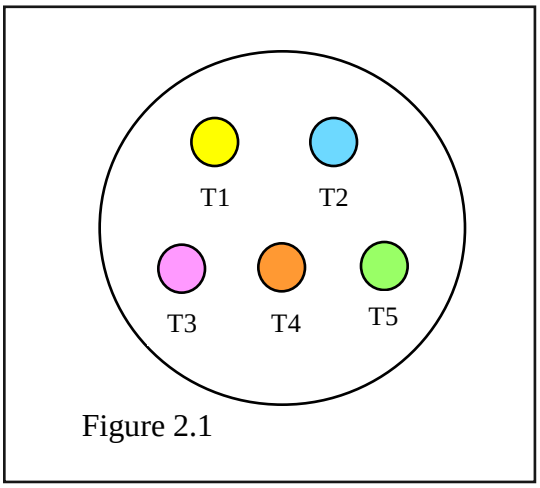

Figure 2.1

1) The day before thawing prepare dishes with culture medium for the thawed embryo(s) and allow to equilibrate in incubator overnight. It is recommended to culture thawed cells in media containing 15-20% HSA or a similar protein, after thawing for 24h before moving to your normal protein concentration.

2) Label one petri dish or 6 well plate: T1, T2, T3, T4, T5. Do this for each straw or device to be thawed. (See Figure 2.1).

3) Pipet 75-100ul of T1, T2, T3, T4, T5 into the labeled dish and cover the drops with mineral oil. (Return media to refrigerator at 2-8°C).

4) Fill container with liquid nitrogen.

5) Select straw(s) or devices to be thawed and place into container with liquid nitrogen.

6) Bring T1 to room temperature (23-27°C).

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I.C.E. EMBRYO THAW PROTOCOL

PART 3 Embryo Thawing

Please Note: All steps are performed at 23-26°C unless otherwise indicated. Use a timer for all steps. Embryo(s) are most often vitrified in a conventional 0.25cc straw or 0.5cc straw, but other micro-volume devices can be used. The cells are located in the middle column of a straw or the tip of the micro-volume device. See Figure 3.1.

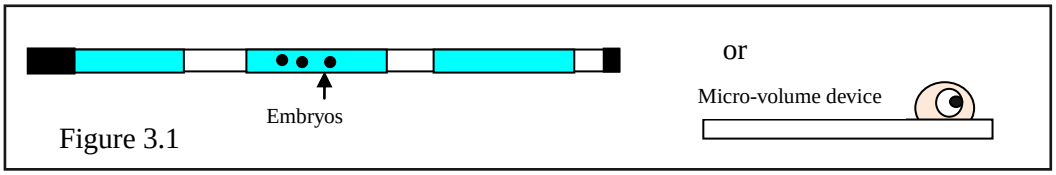

Figure 3.1

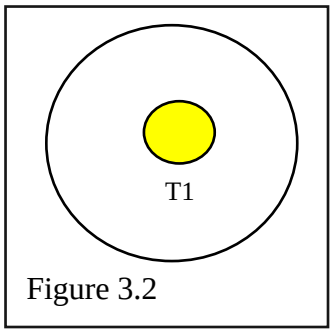

Figure 3.2

For thawing straws:

1) Label one petri dish T1 and pipet 250-300ul T1 into dish. Do not cover with oil. See Figure 3.2.

2) Remove straw from liquid nitrogen and hold for **3 sec in room temperature air**. Use a timer! See Figure 3.4.

3) Submerge straw completely in **37°C water for 7 sec**. Figure 3.4.

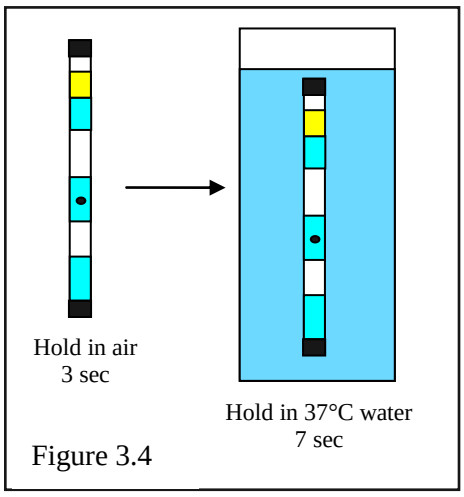

Figure 3.4

4) Wipe excess water off straw and cut off heat-sealed end of the straw. Insert syringe and cut off the other heat-sealed end. See Figure 3.5.

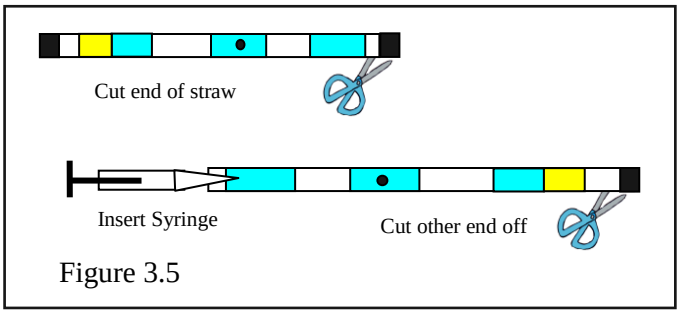

Figure 3.5

5) Empty contents of straw into 250-300ul T1.

6) Shake dish back and forth on workbench for 30-45 sec to mix contents. See Figure 3.6. *This helps dilute out the cryoprotectants and allows the embryo(s) to settle*

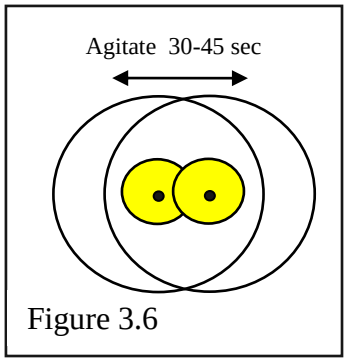

Figure 3.6

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to the bottom of the drop. Be careful not to let the media touch the sides of the dish as this will make locating the embryo(s) more difficult.

Go to Step #7.

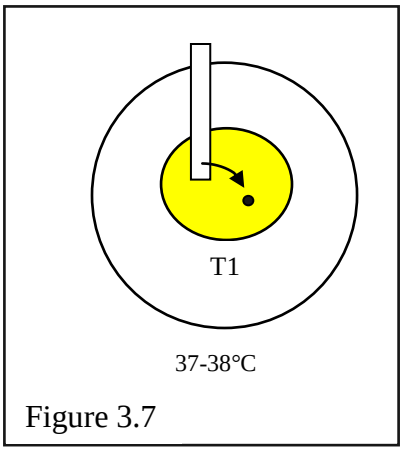

Figure 3.7

For thawing micro-volume devices (cryo-top, cryo-loc, cryo-tec, etc):

1) Label one small petri or organ culture dish T1 and pipet 500ul-1500ul T1 into dish. Do not cover with oil. Place lid on dish. Incubate dish at 37-38°C for 10-15 min to warm the media. Make sure T1 media is warm and at 37-38°C before using.

2) Remove micro-volume device from liquid nitrogen and immediately submerge the end containing the embryo(s) completely into 37-38°C T1. Wait until the embryo(s) fall off the device. See Figure 3.7. Always refer to micro-volume device manufacturers recommendations for thawing.

3) Go to Step #7.

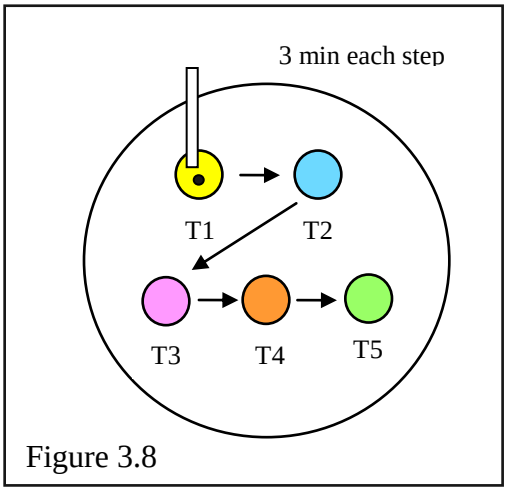

Figure 3.8

All steps are performed at 23-26°C in microdrops under oil.

7) Transfer the embryo(s) to T1 for 3 min. See Figure 3.8.
8) Transfer the embryo(s) to T2 for 3 min.
9) Transfer the embryo(s) to T3 for 3 min.
10) Transfer the embryo(s) to T4 for 3 min.
11) Transfer the embryo(s) to T5 and place dish on a 37°C warmer for 3 min.
12) Transfer the embryo(s) to equilibrated and warmed culture medium in a 37°C incubator.

For optimal performance:

For best results use a new dish (with fresh medium) for each straw thawed.
Store media at 2-8°C.
Culture thawed cells in media containing 15-20% HSA or a similar protein for the first day after thawing.
We suggest culturing the thawed embryos overnight before transfer. This will allow verification of cleavage and further development post-thaw.