Innovative Cryo Enterprises L.L.C.

MICRO_SECURE VITRIFICATION PROTOCOL

Use for vitrification of human cells only.

Vitrification

There are a variety of different devices/methods you can use for vitrifying oocytes, embryos or blastocysts with our media. You can use a standard 0.25cc straw that contains a large volume of media or you can use a device that contains a micro-volume of media such as a cryo-top or cryo-loc or plastic stripper tip ("Micro-secure" method). This protocol briefly describes the micro-secure method.

Micro-Secure Method:

1) Prepare the cells by incubation in the appropriate vitrification media.
2) Prepare a stripper tip for loading by cutting 3cm of the large end off that attaches to the pipet holder. This shortens the tip for loading into a standard 0.5cc straw or 0.3cc CBS straw.
3) Load the cell(s) into the tip of the plastic stripper tip. First load 2 ul of V3 vitrification media then load the cells in the last 1ul of media. (See diagram below).
4) Wipe off the end of the stripper tip with a sterile cloth/gauze. This will remove any excess media from the outside of the pipet tip and prevent sticking to the 0.5cc container straw.
5) Insert the stripper tip into a blank straw. If you use the 0.3cc CBS straw you can heat seal an Identification rod into the small part of the straw.
6) Heat seal the open ends of the straw.
7) Hold the straw vertically so that the stripper tip is standing upright inside the straw.
8) Plunge and store in LN_2.

For optimal performance:

Follow the appropriate protocol for incubation times in vitrification solutions.

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.

Store media at 5°C.

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Thawing:

Micro-Secure Method:

1) Locate the straw to be thawed and grasp the bottom with forceps keeping the straw submerged in LN2. Lift the top part of the straw above the level of LN2. Typically a CBS embryo straw is used that has a plastic plug 1/3rd of the way down. The stripper tip is located in the lower portion of the straw. Cut off the top of the straw just below the plastic plug and above the top of the stripper tip.

Prior to thawing, grasp the top of the straw with forceps keeping in submerged in LN2. Tap the straw against the side of the container and listen for it to rattle around in the carrier straw. This will ensure that it falls out immediately into the thaw medium.

2) Remove the straw from LN2 and immediately "pour out" the stripper tip containing the cells into a large drop (around 400ul, use an organ culture dish with the center well filled or a 4-well dish) of T1-media at 37-38°C. Hold the stripper tip so that the end with the cells is completely submerged in the thaw medium for at least 3 seconds to ensure complete thawing. Carefully attach a stripper pipet or rubber bulb and gently expel the cells out of the tip into the T1 media.

Transfer cell(s) to (50ul-75ul microdrops under oil):

3) T1-media	2 min at 23-26°C
4) T2-media	3 min at 23-26°C
5) T3-media	3 min at 23-26°C
6) T4-media	3 min at 23-26°C
7) T5-media	3 min on 37°C place on warmer
8) Equilibrated, warmed culture medium	37°C incubator

For optimal performance:

For best results use a new dish (new medium) for each straw thawed.

Store media at 5°C.

Culture thawed cells in media containing around 20% HSA or a similar protein after thawing and prior to transfer.

After thawing, you can transfer the blastocyst(s) at any time. If the cells are intact and translucent it is alright, but not necessary to wait several hours for re-expansion.

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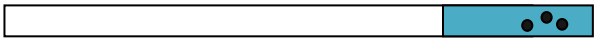
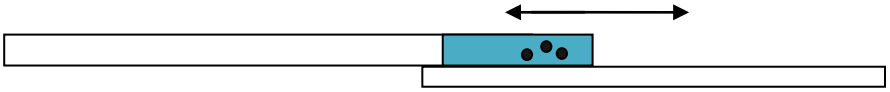
Vitrification: Micro-Secure Method

1) Load cells into stripper tip. First load 2ul media and then cells (1-5). Note: if the stripper tip is too long you can shorten it by cutting off 3cm from the end that attaches to the holder.

Stripper tip



2) Wipe off tip of stripper on sterile cloth to remove any media from the outside of the pipet tip.



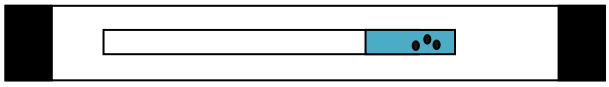
sterile cloth

3) Heat seal one end of a blank 0.5cc straw.

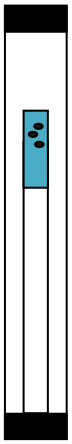
Heat-seal

4) Insert stripper tip with cells carefully into the 0.5cc straw.

4) Heat-seal the open end.



5) Stand upright and store in liquid nitrogen.



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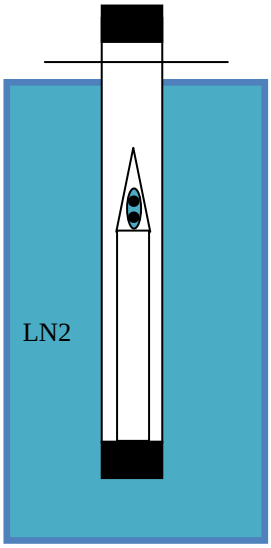
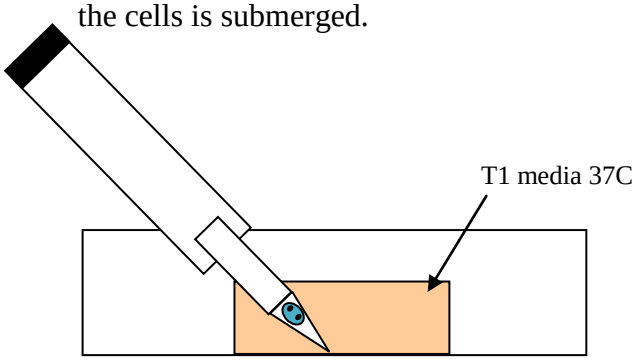
Thawing: Micro-Secure Method

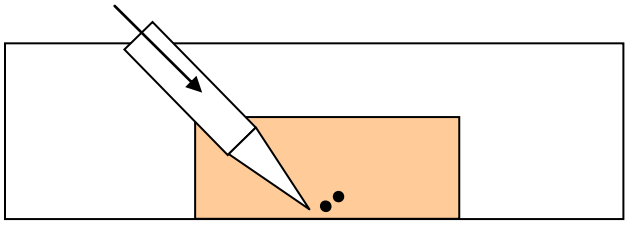
1) Raise top of straw above LN2 and cut off top below heat seal. Use forceps to hold straw in LN2.



2) Tip straw so that the stripper tip falls out into a large drop of T1 medium at 37°C. An organ culture or 4-well dish works nicely. Make sure the entire tip containing the cells is submerged.

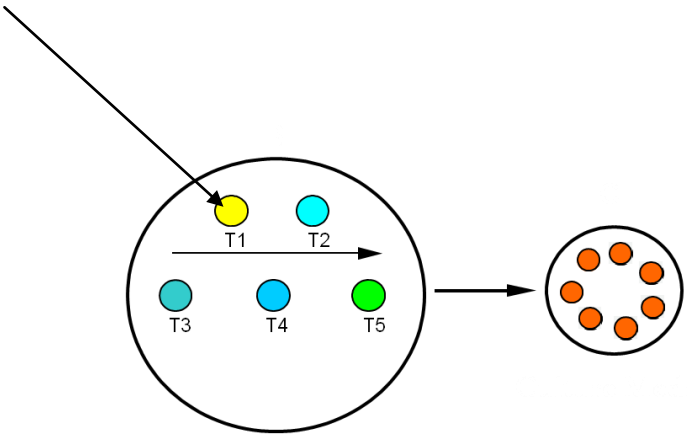


3) Attach a stripper pipet, or rubber bulb to expel the cells into the T1 media. Follow the appropriate dilution protocol for the cell type (oocytes, embryos, or blastocysts) you are vitrifying.



Transfer cells to:

T1	2 min	23-26°C
T2	3 min	23-26°C
T3	3 min	23-26°C
T4	3 min	23-26°C
T5	3 min	37C
Culture media		37C



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