Innovative Cryo Enterprises L.L.C.

BLASTOCYST VITRIFICATION PROTOCOL

Use for vitrification of blastocysts only. Do not freeze cleavage stage embryos or eggs.

Blastocysts with a large cavity & thinning zona or are hatching/hatched can be frozen.

Blastocyst Vitrification

1) V1-Blast 5 min at 23-26°C
2) V2-Blast 5 min at 23-26°C
3) V3-Blast 1-2 min at 23-26°C

Transfer blastocyst(s) into V3-Blast with minimal carry-over & load into straw or on device.

Be sure to give the blastocysts at least 1min in the V3 prior to plunging into LN2.

4) Load blastocyst(s) in straw between columns of V3-Blast. See Figure below. *Can use micro-volume device.*

Aspirate the first column of V3 to the cotton plug, as this will allow the columns to remain intact.

5) Heat seal both ends of the straw. *You must heat-seal. Periodically check seal under microscope to ensure sealer is working properly.*
6) Plunge and store in LN_2.

For optimal performance:

Push the cotton plug in 2cm 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.

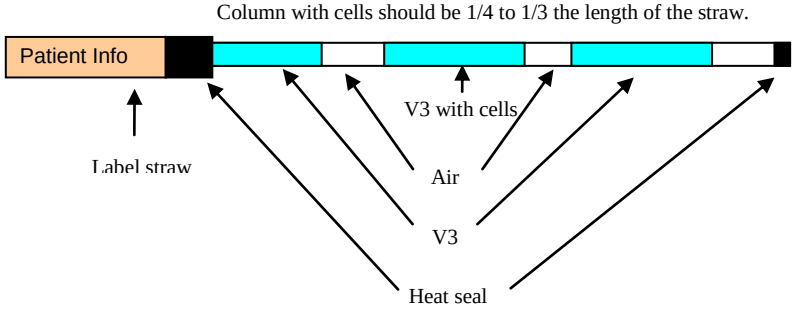
Wait until the blastocoel cavity is expanding and the zona is thinning to vitrify.

There is no need to collapse the blastocoel prior to vitriifcation.

Store media at 5°C.

Straw Loading Diagram

Conventional 0.25cc straws (with a 0.5cc straw attached as a label) or 0.3cc CBS straws can be used.



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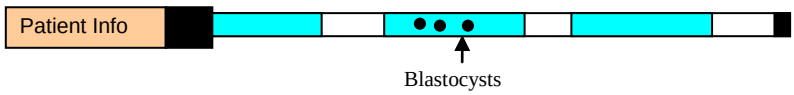
Blastocyst Thawing

Please Note: Blastocysts are most often vitrified in a conventional 0.25cc straw or 0.3cc CBS straw, but other devices can be used. The cells are located in the middle column. See diagram below.



Blastocyst Thawing Protocol:

1) Thaw straw 6 sec in room temperature air and then 10 sec in 30°C water. *Pull straw out of tank slowly to hold in air. Move straw around in the water bath, make sure straw is completely submerged.*

2) Quickly wipe straw and cut off top heat-sealed end of straw, insert syringe and cut off other sealed end.

3) Empty contents of straw into 300ul of T1- Blast in a 60mm dish.

4) Shake dish on workbench for 30 sec to mix contents.

This helps dilute out the cryoprotectants and allows the blastocysts to settle 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 blastocyst(s) more difficult.

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

5) T1- Blast	3 min at 23-26°C
6) T2- Blast	3 min at 23-26°C
7) T3- Blast	3 min at 23-26°C
8) T4- Blast	3 min at 23-26°C
9) T5- Blast	3 min on a 37°C warmer
10) 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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