

RAPID-i™ VITRIFICATION OF BLASTOCYSTS

Vitrification may only be performed by staff trained in vitrification procedures.

Incorrect handling or sealing of the RapidStraw™ can cause a pressure build up inside that may result in damage or even explosion of the straw during the warming procedure



Rapid-i™ kit



SmartBox™



Rapid-i™ Sealer



Rapid-i™ Cryocane with goblet



RapidVit™ Blast kit

All procedures should be performed at +37°C

1. Fill the SmartBox with liquid nitrogen up to 1 cm from the box's rim and place the lid on top of the box.

Always maintain a sufficient level of liquid nitrogen in the SmartBox

2. Place 0.5-1 ml of each of the following solutions into separate wells of a 5-well plate and warm to

37°C

Vitri 1™ Blast

Vitri 2™ Blast

Vitri 3™ Blast



3. Label the exact number of RapidStraws needed with the patient's identification. Place the label below the top, black mark of the straw.



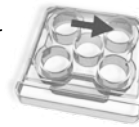
4. Collapse the blastocysts by inserting an ICSI-pipette, or by using a laser beam.

5. Transfer the collapsed blastocysts from culture into Vitri 1 Blast and let the blastocysts remain in the solution for



Vitri 1 Blast 5-20 min

6. Move an appropriate number of blastocysts into Vitri 2 Blast. The blastocysts remain in this solution for

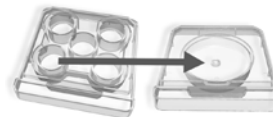


Vitri 2 Blast 2 min

7. Place the RapidStraw in liquid nitrogen. Make sure that the RapidStraw is securely attached to the magnet in the SmartBox.



8. When 30 sec remain, make a 20 µl droplet of Vitri 3 Blast on a non-toxic surface, preferably a 40 mm culture dish.



9. When 10 sec remain, begin collecting the blastocysts. Transfer the blastocysts into the 20 µl droplet of Vitri 3 Blast and let them remain in this solution for 45 sec, including the time it takes to load Rapid-i and vitrify.

Remove the stainless steel rod from the RapidStraw after 15 seconds have elapsed and discard.

Vitri 3 Blast 45 sec



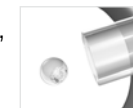
10. Place Rapid-i on the microscope stage with the flat side down. Locate the correct plane of focus so that the hole of Rapid-i is in view, for easy loading.

11. Collect the blastocysts with a Vitrolife micro-pipette. Keep the blastocysts close together at the end of the pipette.



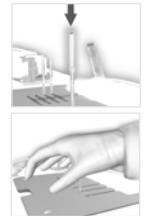
Avoid overfilling the hole or else the blastocysts may float out

12. Slide the hole of Rapid-i into view, in the microscope. Move the tip of the pipette close to the



wall of the hole in Rapid-i and expel the blastocysts into the hole.

13. Quickly place Rapid-i vertically into the pre-cooled straw sitting in the Smartbox. Cover the hole immediately after insertion for a few seconds to prevent that Rapid-i accidentally pops out.



14. Immediately seal the top of the RapidStraw using the Rapid-i Sealer. Inspect the seal to ensure that sealing was correctly performed. Place the storage vessel (e.g. cryocane with attached goblet) into the SmartBox.



15. Move the sealed RapidStraw from the lid and into the goblet, so that the RapidStraw with the blastocysts does not leave the liquid nitrogen.

16. Transfer the cryocane with goblet to long term storage.

RAPID-i™ WARMING OF BLASTOCYSTS



SmartBox™



Rapid-i™ forceps



Rapid-i™ cutter



RapidWarm™ Blast kit

1. Place the Smartbox on the lab bench close to the microscope and fill it with liquid nitrogen up to 1 cm from the box's rim and place the lid on top of the box.

2. Place 0.5-1 ml of each of the following media into separate wells of a 5-well plate and warm to

37°C

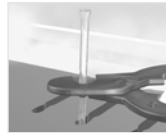
Warm 1™ Blast
Warm 2™ Blast
Warm 3™ Blast



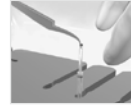
3. Move the cryocane and goblet containing the RapidStraws™ into the liquid nitrogen in the SmartBox.

4. Without leaving the liquid nitrogen, remove one RapidStraw from the goblet and place it in a slit of the lid.

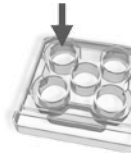
5. Warm the RapidStraw with your fingers around the black mark to get a better view of the black tab on Rapid-i. Hold the RapidStraw well above the black mark and use the Rapid-i cutter to cut the RapidStraw 3 mm above the back end of Rapid-i. Do not lift the RapidStraw from the lid and make sure it stays up-right in liquid nitrogen.



6. Lift Rapid-i (using Rapid-i forceps) out of the RapidStraw just enough to enable you to grasp the end with your finger tips. Then quickly (preferably less than 2 seconds), but carefully, remove Rapid-i from the RapidStraw and plunge the tip and hole of Rapid-i into the Warm1 Blast solution.



7. Allow the blasto-cysts to fall from the device and sink to the bottom. Leave for



Warm 1 Blast 2 min

8. Transfer the blastocysts into Warm 2 Blast and let the blastocysts remain in the solution for



Warm 2 Blast 3 min

9. Transfer the blastocysts into Warm 3 Blast and let the blastocysts remain in the solution for



Warm 3 Blast 5-10 min

10. Rinse the blastocysts in culture media several times and continue culture according to laboratory practice.



11. Discard the used Rapid-i.