

RAPID-i™ VITRIFICATION OF CLEAVAGE STAGE EMBRYOS

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™ Cleave 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™ Cleave

Vitri 2™ Cleave

Vitri 3™ Cleave



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

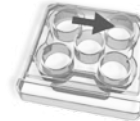


4. Transfer the embryos from culture into the Vitri 1 Cleave and let the embryos remain in the solution for



Vitri 1 Cleave 5-10 min

5. Move an appropriate number of embryos into Vitri 2 Cleave. The embryos remain in this solution for

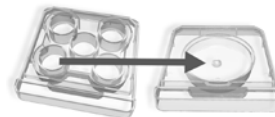


Vitri 2 Cleave 2 min

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



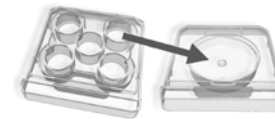
7. When 30 sec remain, make a 20 µl droplet of Vitri 3 Cleave on a non-toxic surface, preferably a 40 mm culture dish.



8. When 10 sec remain, begin collecting the embryos from Vitri2 Cleave. Transfer the embryos into the 20 µl droplet of Vitri 3 Cleave and let them remain in this solution for 30 sec, including the time it takes to load Rapid-i and vitrify.

Remove the stainless steel rod from the RapidStraw and discard.

Vitri 3 Cleave 30 sec



9. 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.



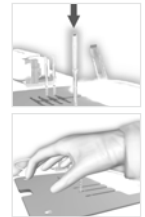
10. Collect the embryos with a Vitrolife micropipette. Keep the embryos close together at the end of the pipette.



11. 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 embryos into the hole.

Avoid overfilling the hole or else the blastocysts may float out

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



13. 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.



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

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

RAPID-i™ WARMING OF CLEAVAGE STAGE EMBRYOS



SmartBox™



Rapid-i™ forceps



Rapid-i™ cutter



RapidWarm™ Cleave

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

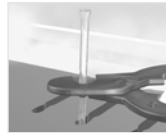
Warm 1™ Cleave
Warm 2™ Cleave
Warm 3™ Cleave
Warm 4™ Cleave



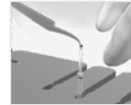
3. Move the cryocane and goblet containing the RapidStraws™ into the liquid nitrogen in the SmartBox.

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

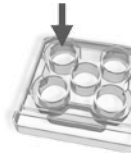
5. Warm the RapidStraw with your fingers around the black mark to get a better view of the black tab on Rapid-i. Hold the RapidStraw well above the black mark and use the Rapid-i cutter to cut the RapidStraw 3 mm above the back end of Rapid-i. Do not lift the RapidStraw from the lid and make sure it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

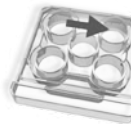


7. Allow the embryos to fall from the device and sink to the bottom. Leave for



Warm 1 Cleave 10-30 sec

8. Transfer the embryos into Warm 2 Cleave and let the embryos remain in the solution for



Warm 2 Cleave 1 min

9. Transfer the embryos into Warm 3 Cleave and let the embryos remain in the solution for



Warm 3 Cleave 2 min

10. Transfer the embryos into Warm 4 Cleave and let the embryos remain in the solution for



Warm 4 Cleave 5 min

11. Rinse the embryos in culture media several times and continue culture according to laboratory practice.



12. Discard the used Rapid-i.