Rapid-i vitrification of cleavage stage embryos

For detailed information, consult the Instructions for use for RapidVit Cleave, RapidWarm Cleave and Rapid-i Kit

Vitrification should only be performed by **staff trained** in vitrification procedures. Ensure you follow the protocol precisely.

The **timeframes** are critical. The recommended **volumes** should not be changed. Volume changes will affect temperature control as well as osmolality, which may give suboptimal results. All procedures should be performed on a heated stage **(solutions at 37 °C)** and ambient atmosphere.

Step 1 Prepare


RapidVit™ Cleave


Rapid-i™ Kit


SmartBox™


Vitrolife 5-well culture dish


Rapid-i™ Sealer

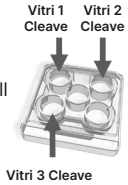

Rapid-i™ CryoCane

Rapid-i™ Goblet

Fill the SmartBox with liquid nitro-gen 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.

Place 0.5-1 ml of each Vitri Cleave solution into separate wells of a Vitrolife 5-well culture dish. Place the lid on and warm to 37 °C.



Do not place the Vitri Cleave solutions in a CO2 incubator.

37 °C

Label all RapidStraws to be used with the patient's identification between the black marks.

Step 2 Expose to Vitri 1 Cleave

Transfer the embryos into Vitri 1 Cleave and leave for 5–10 minutes.

Vitri 1 Cleave: 5–10 minutes

Step 3 Expose to Vitri 2 Cleave

Before moving an appropriate number of embryos, prime the micro-pipette with Vitri 2 Cleave.

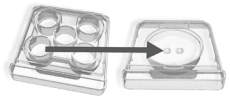
Pick up the embryos with minimal volume to avoid dilution.

Transfer the embryos into Vitri 2 Cleave and ensure complete exposure. Keep the lid on whenever applicable.

Vitri 2 Cleave: 2 minutes

After moving the embryos to Vitri 2 Cleave, place the RapidStraw with metal rod in the SmartBox to cool down.

When less than 30 seconds of the 2 minutes remain: prepare two 50 µl droplets of Vitri 3 Cleave on a Vitrolife culture dish.

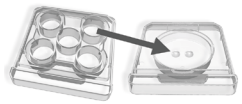


Step 4 Expose to Vitri 3 Cleave

Prime the micro-pipette with Vitri 3 Cleave.

Pick up the embryos with minimal volume to avoid dilution.

Transfer the embryos into to the first Vitri 3 Cleave droplet.



Empty the micro-pipette outside the droplet and prime again from the second droplet.

Immediately transfer the embryos to the second Vitri 3 Cleave droplet.

The total exposure time, from entering Vitri 3 Cleave until vitrification, should be 30 seconds.

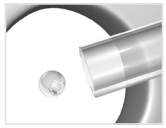
Vitri 3 Cleave: 30 seconds

Step 5 Load the Rapid-i

Before loading the embryos on the Rapid-i, remove the metal rod from the RapidStraw and discard. Position the Rapid-i next to the Vitri 3 Cleave droplets.

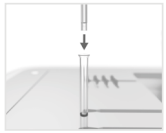
Collect the embryos with the micro-pipette. Keep them close together at the tip of the pipette.

Load the embryos into the Rapid-i hole, without overfilling or underfilling.



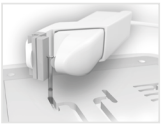
Step 6 Vitrify and seal

Immediately after loading, place the Rapid-i into the pre-cooled RapidStraw in the SmartBox.



Cover the RapidStraw opening with your hand for a few seconds to prevent that the Rapid-i pops out.

Seal the top of the RapidStraw using the Rapid-i Sealer.



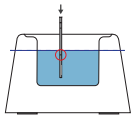
Inspect the seal to ensure that sealing was correctly performed.

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.

Step 7 Store

Place the Rapid-i CryoCane with attached Rapid-i Goblet in the SmartBox.

Move the RapidStraw into the Rapid-i Goblet. The lowest black mark of the RapidStraw must always be submerged in liquid nitrogen.



Transfer to long term storage according to laboratory practice.

The sealed RapidStraw may never be removed from liquid nitrogen.

Pre-caution: Discard product if bottle integrity is compromised. Do not use if solution appears cloudy. Ensure Rapid-i Kit packaging is intact and use by date has not expired. Ensure Rapid-i is intact before starting procedure.

Vitrolife

Rapid-i warming of cleavage stage embryos

For detailed information, consult the Instructions for use for RapidVit Cleave, RapidWarm Cleave and Rapid-i Kit

The **timeframes** are critical. The recommended **volumes** should not be changed. Volume changes will affect temperature control as well as osmolality, which may give suboptimal results. All procedures should be performed on a heated stage **(solutions at 37 °C)** and ambient atmosphere.

Step 1 Prepare

RapidWarm™ Cleave Kit

SmartBox™

Vitrolife™ 5-well culture dish

Rapid-i™ Cutter

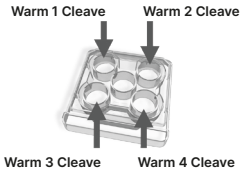
Rapid-i™ Forceps

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.

Place 0.5-1 ml of each Warm Cleave solution into a Vitrolife 5-well culture dish.

Place the lid on and warm to 37 °C.

Do not place the Warm Cleave solutions in a CO2 incubator.



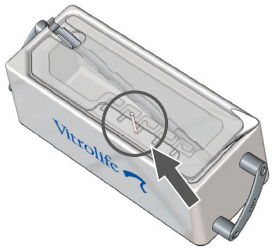
37 °C

Step 2 Move RapidStraw to SmartBox

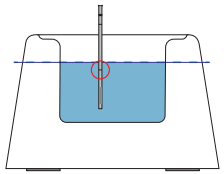
Collect the embryos from long term storage.

Place the Rapid-i CryoCane with Rapid-i Goblet and the RapidStraw in the SmartBox.

Remove the RapidStraw from the Rapid-i Goblet and place it in the slit of the lid where the magnet is located.



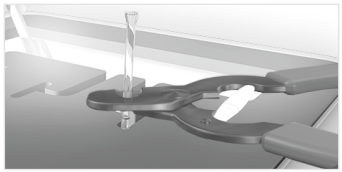
The lowest black mark of the RapidStraw must always be submerged in liquid nitrogen.



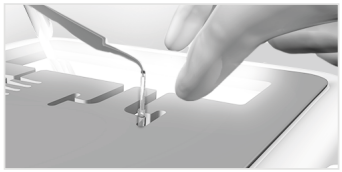
Step 3 Open RapidStraw

Hold the RapidStraw with your fingertips or Rapid-i Forceps.

Cut the RapidStraw just above the black tab of the Rapid-i, using the Rapid-i Cutter.



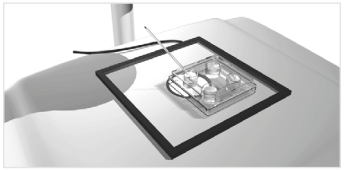
Lift the Rapid-i out of the RapidStraw using the Rapid-i Forceps, just enough to grasp the end with your fingertips.



Ensure that RapidStraw is opened by cutting, before it is removed from liquid nitrogen.

Step 4 Expose to Warm 1 Cleave

Quickly (within 1 second) but carefully, move the Rapid-i and plunge it into Warm 1 Cleave.



Verify that the embryos are released in the medium and then remove the Rapid-i.

The total exposure time, from plunging the samples into Warm 1 Cleave, should be 10-30 seconds.

Warm 1 Cleave: 10-30 seconds

Step 5 Expose to Warm 2 Cleave

Pick up the embryos with minimal volume to avoid dilution.

Transfer the embryos into Warm 2 Cleave and leave for 1 minute.

Warm 2 Cleave: 1 minute

Step 6 Expose to Warm 3 Cleave

Pick up the embryos with minimal volume to avoid dilution.

Transfer the embryos into Warm 3 Cleave and leave for 2 minutes.

Warm 3 Cleave: 2 minutes

Step 7 Expose to Warm 4 Cleave

Pick up the embryos with minimal volume to avoid dilution.

Transfer the embryos into Warm 4 Cleave and leave for 5 minutes.

Warm 4 Cleave: 5 minutes

Rinse the embryos in culture medium several times.

Culture according to laboratory practice.

Pre-caution: Discard product if bottle integrity is compromised. Do not use if solution appears cloudy.

Vitrolife