

# Rapid-i vitrification of blastocysts

For detailed information, consult the Instructions for use for RapidVit Blast, RapidWarm Blast 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.

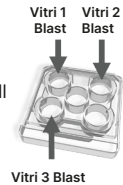
## Step 1 Prepare



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 Blast 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 Blast solutions in a CO2 incubator.

37 °C

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



Blastocysts may be collapsed prior to vitrification with a laser or an ICSI pipette.

## Step 2 Expose to Vitri 1 Blast

Transfer the blastocysts into Vitri 1 Blast and leave for 5-20 minutes.



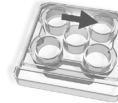
Vitri 1 Blast: 5-20 minutes

## Step 3 Expose to Vitri 2 Blast

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

Pick up the blastocysts with minimal volume to avoid dilution.

Transfer the blastocysts into Vitri 2 Blast and ensure complete exposure. Keep the lid on whenever applicable.

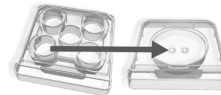


Vitri 2 Blast: 2 minutes

After moving the blastocysts to Vitri 2 Blast, 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 Blast on a Vitrolife culture dish



## Step 4 Expose to Vitri 3 Blast

Prime the micro-pipette with Vitri 3 Blast.

Pick up the blastocysts with minimal volume to avoid dilution.

Transfer the blastocysts into the first Vitri 3 Blast droplet.



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

Immediately transfer the blastocysts to the second Vitri 3 Blast droplet.

The total exposure time, from entering Vitri 3 Blast until vitrification, should be 45 seconds.

Vitri 3 Blast: 45 seconds

## Step 5 Load the Rapid-i

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

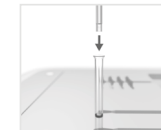
Collect the blastocysts with the micro-pipette. Keep them close together at the tip of the pipette.

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



## Step 6 Vitrify and seal

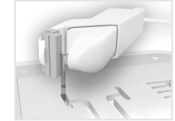
Immediately after loading, place the Rapid-i into the pre-cooled RapidStraw in the SmartBox.



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.

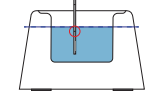
Incorrect handling or sealing of the RapidStraw can cause a pressure build up inside that may result in damage or even explosion of the straw during the warming procedure.

## 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.

# Rapid-i warming of blastocysts

For detailed information, consult the Instructions for use for RapidVit Blast, RapidWarm Blast 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

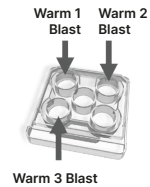


Fill the SmartBox with liquid nitrogen up to 1 cm from the box's rim and place the lid on top of the box. Always maintain a sufficient level of liquid nitrogen in the SmartBox.

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

Place the lid on and warm to 37 °C.

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



37 °C

## Step 2 Move RapidStraw to SmartBox

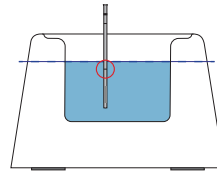
Collect the blastocysts from long term storage.

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.



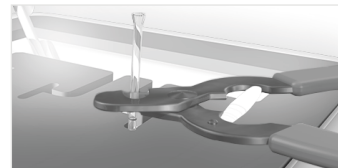
The lowest black mark of the Rapid-Straw must always be submerged in liquid nitrogen.



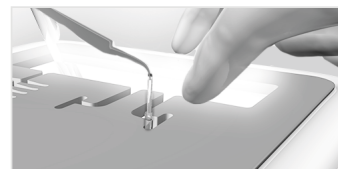
## Step 3 Open RapidStraw

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.



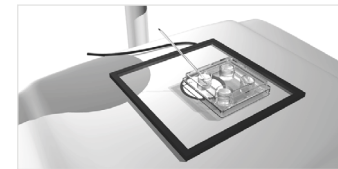
Lift the Rapid-i out of the RapidStraw using the Rapid-i Forceps, just enough to grasp the end with your fingertips.



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

## Step 4 Expose to Warm 1 Blast

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



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

The total exposure time, from plunging the samples into Warm 1 Blast, should be 2 minutes.

Warm 1 Blast: 2 minutes

## Step 5 Expose to Warm 2 Blast

Pick up the blastocysts with minimal volume to avoid dilution.

Transfer the blastocysts into Warm 2 Blast and leave for 3 minutes.



Warm 2 Blast: 3 minutes

## Step 6 Expose to Warm 3 Blast

Pick up the blastocysts with minimal volume to avoid dilution.

Transfer the blastocysts into Warm 3 Blast and leave for 5-10 minutes.



Warm 3 Blast: 5-10 minutes

Rinse the blastocysts in culture medium several times.

Culture according to laboratory practice.

