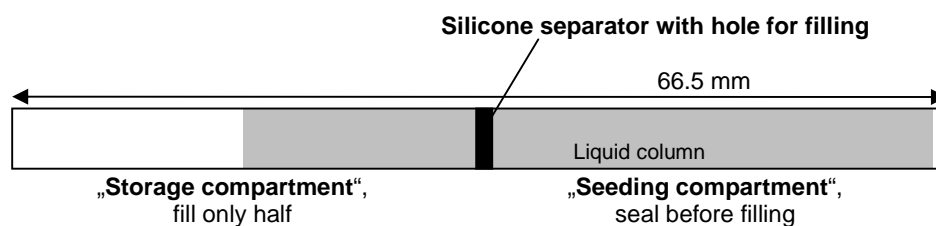


Quick guide on CTE security straws for tissue

1. Preparing the straws

Before loading the straws with tissue they have to be filled with cryoprotective solution, avoiding any air bubbles. Use a sterile, toxicity tested syringe with a long (min. 60 mm), sterile cannula. Make sure the cryoprotective solution has been mixed thoroughly before use! This is mandatory to obtain satisfactory results.

- a) Seal one end of the tissue straw, using a heat sealer with extra wide sealing jaws (prod. no. 13135/0100).
- b) Attach the cannula to the syringe and fill the syringe with cryoprotectant.
- c) Make sure there is no air left inside the syringe / cannula by releasing some droplets of cryoprotectant from the cannula.
- d) Carefully insert the cannula into the open end of the straw, then fit the cannula through the hole of the silicone separator. Carefully fill the lower compartment of the straw with cryoprotectant, starting at the bottom of the compartment. Make sure there are no air bubbles left in the cryoprotectant as they might impair ice growth. Shifting the silicone plug with the cannula should also be avoided as this might alter the progression of crystallization.
- e) Leave the upper half of the storage compartment empty. This space is needed for pressure compensation during freezing and for thermal insulation during heat sealing.
- f) Handle the straws carefully and store in a sterile Petri dish until loading them with tissue.



2. Loading the straws with tissue

Use fine tweezers, Eppendorf pipette tips (negative pressure) or similar tools for handling tissue pieces and loading the straws. It is common practice to store one piece of tissue per straw.

Important: make sure that

- **the piece of tissue is always surrounded by cryoprotectant, i.e. never blocks the complete diameter of the tissue straw**
- **the piece of tissue is not obstructing the hole in the silicone plug, preventing ice from passing through the plug**

Heat seal the storage compartment after loading it with tissue. The empty space will prevent the tissue from warming up during sealing. Carefully insert the tissue straws into the respective straw holders of the CTE controlled rate freezer, seeding compartment down, storage compartment up.

3. Labelling the tissue straws

Wrap self-adhesive, cryo-proof labels around the lower part of the straw (corresponding to the seeding compartment). This will keep the storage compartment accessible for visual control. There is no need to label the reference straw covering the temperature sensor.

4. How to handle the heat sealer

- a) Inspect the jaws of the welding tongs in regular intervals. The jaws of the heat sealer can be changed after loosening the small hexagon sockets. Before using the welding tongs of the heat sealer, make sure that the welding jaws are in parallel orientation (i.e., they must not be wedged) and the hexagon sockets are tightened. The welding jaws must close without gap when pressed against each other! Otherwise the welding tongs might not be able to properly seal straws.
- b) Inspect the silicon protection of the welding jaws
The silicon protection must protrude the welding jaws by approx. 1 mm! Otherwise the welding tongs might burn holes into the straws or the straws and the welding jaws might stick together. Keep the silicon covers clean by removing media remnants with a paper towel moistened with 70 % alcohol or water immediately after sealing. The silicon covers have to be changed from time to time.

- c) Sealing temperature should be set to 185 – 190°C to seal CTE straws reliably. To change sealing temperature, press the “↓” button once until the “o” symbol in the display starts blinking, then change temperature by using “+” and “-“ buttons.
- d) Sealing straws
Hold the welding tongs horizontally and place the end of a straw centrally on one of the welding jaws. Then press the welding tongs and hold for approx. 5 sec (count in your head). The welding seams should be approx. 2 mm long.
- e) Inspect the welding seams
Practice on straws without cells but filled with liquid before switching to routine sealing! Appropriate welding seams should be smooth and transparent and lack any inclusions or channels. Check under the stereo microscope!
- f) The welding seams of all straws should be similar in length to maintain equal cooling conditions for all straws.

5. Recommended freezing program for ovarian cortex

Once a month and after changing the Pt100 temperature sensor, an auto-zero calibration has to be performed to ensure reliable operation of your CTE controlled rate freezer (see manual).

Program:

| Ramp | Cooling rate (°C/min) | Final temperature (°C) |
|------|-----------------------|------------------------|
| 1 | - 5.0 | 4 |
| 2 | - 1.0 | - 1 |
| 3 | - 0.3 | - 34 |
| 4 | - 50 | - 190 |

(Total duration of this temperature profile: 121 min)

Please note: when watching ice crystal formation inside the straws, do not mistake frost formation on the outer surface of the straws for ice growth! Frost formation might mimic fast ice growth or even subsequent thawing whereas real ice formation will progress slowly and continuously. If necessary, use a magnifying glass to identify real ice growth.

Reference straw and reference straw holder

Always use a sperm straw (66.5 mm, no plug, prod. no. 19044/9010) as reference straws. Seal this straw on one end, then fill with cryopreservation solution using a cannula. Avoid any air bubbles inside the reference straw. Carefully (!) insert the

cone of the temperature sensor into the upper end of the reference straw. **Avoid deflecting the temperature sensor from its longitudinal axis as it is coated by glass and sensitive towards breakage!** Spilling cryopreservation solution has to be removed from the outside of the reference straw (paper towel) as it might disturb temperature measurement. Use a new reference straw for each cryopreservation procedure.

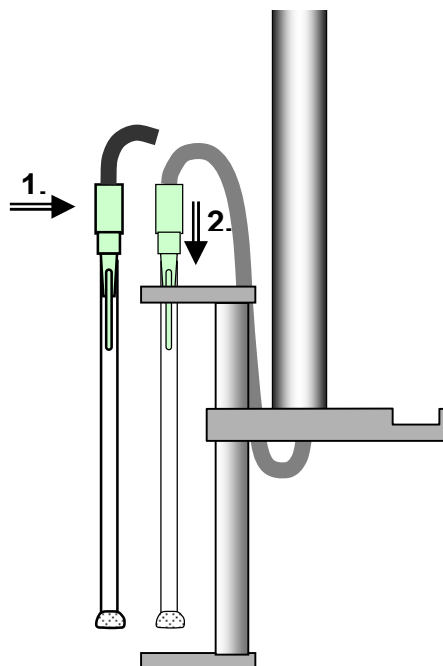


Caution: The platinum sensing resistor is coated with glass (diameter 0.9 mm) and is highly sensitive towards any mechanical impact!

Hold the temperature sensor and attached reference straw vertically. The cone of the temperature sensor must be above the reference straw holder which is vertically oriented. The welding seam of the reference straw must be correctly oriented to fit the slot of the lower part of the reference straw holder (see figure). Insert the reference straw into the upper part of the straw holder from the side, then carefully move downwards and plug the cone of the temperature sensor into the drill hole of the holder. Simultaneously, the welding seam of the reference straw must fit its slot. Do not try to latch the cone of the temperature sensor from the side!



Do not try to latch the cone of the temperature sensor from the side. Danger of breakage!



**Inserting the reference straw:
two steps!**

The reference straw holder can be turned by 90° as both auto-zero calibration and seeding point determination (see manual) have to be performed with special straws (EC-02; rectangular shape with “tail”). To turn the reference straw holder, do not loosen the screw of the swivel joint! It is sufficient to just pull the holder by approx. 1 mm and turn it by 90°. A spring mechanism will arrest it in the new position. Do not turn the reference straw holder when the temperature sensor is attached!

When rectangular straws and horizontal holder position are used, the straws are attached to the holder in the same way as shown above. Make sure that the “tail” of the straw is vertically oriented.

6. Handling and storage of frozen tissue straws

Make sure that the straws are never warmed to temperatures above -130°C after completing a freezing program. Use the CTE handling unit to pack the straws without warming. We recommend to use triangular cassettes or NUNC cryo boxes (75 mm height) to pack the straws for long term storage. Cassettes can be stored in conventional canisters of liquid nitrogen storage tanks.

7. Auto-zero calibration of the Pt 100 sensor

Please note: For auto-zero calibration of the Pt 100 sensor, special straws with 90° “tail” have to be used! For this purpose, the reference straw holder has to be turned by 90° (to horizontal position).

In the new security straws, ice crystal formation is accurately controlled. Consequently, the heat of crystallization will rapidly dissipate, there will be no heat peak which is used by the machine to determine the zero point.

Please note:

MTG Medical Technology Vertriebs-GmbH and CTE Cryo Technik Erlangen GmbH have established the procedures described above in all conscience. Nevertheless, we strongly recommend to practice all steps extensively on spare or animal tissue before integrating them into clinical routine.

We strongly advise you to run tests on both the new freezer and associated procedures before cryopreserving valuable tissue!

According to individual lab setups or personal preferences, slight modifications to the procedures described above might be beneficial. MTG and CTE assume no liability with regard to any adverse effects or damage related to the use of this quick guide.