

Heat Sealing

Although heat-sealing cryo straws properly may seem easy, it can be a major source of problems, even for the experienced lab. Please review this material to ensure you are getting good heat-seals.

Exploding straws are the result of faulty heat-sealing or a crack or break in the straw itself. There are 2 basic faults when heat-sealing. The first is not using a hot enough setting on the sealer. This can be easily corrected. The second is damaging the straw during heat-sealing, sometimes by using too much heat. This is the only way a straw will explode.

Liquid nitrogen seeps inside the straw through a faulty seal or crack and then expands quickly upon warming the straw. If the crack is large enough the liquid nitrogen will simply shoot out the opening. The end result is that the cells inside the straw are usually lost or dead.

This occurs by not heat-sealing with enough heat to melt the straw together. Upon rapid cooling by plunging into liquid nitrogen, the plastic straw shrinks rapidly and can separate, in part or along the entire seal.

Heat Sealer

We have tested numerous heat sealers and have found AIE hand impulse sealers to be a superior product. When looking for a heat-sealer you should consider the following:

- A wide heating element; 5mm rather than the 2mm thin wires in most sealers.
- Temperature control that goes well beyond what is needed. This will allow sealing of a variety of straws or plastic materials.
- A compact, well built, easy to maintain, reliable machine that gives reproducible results year after year.



AIE-205

The AIE-205 is a 120 volt AC hand impulse sealer. It also comes in a 220 Volt model. There is also the **AIE-105T**, which is 4" long rather than 8", so it is smaller and costs less! It is a well made machine that will give years of service. This is the one ICE recommends. And it is under \$70!

Ordering:

You can order the **AIE-105T** online: http://www.pack-secure.com/AIE-105T--Hand-Impulse-Bag-Sealer-4-Inch-Long-5-mm-Wide-Seal- p_1297.html. Scroll to the bottom of the page to order.

The sealer comes with an extra heating element and teflon pad. Regular cleaning with an alcohol swab will ensure years of problem-free seals.

Straws

There are 2 basic types of 0.25cc straws. IMV-type straws and CBS (Cryo Bio System) straws. Either one will work well with the I.C.E. vitrification system.

- 1) The IMV straws can be purchased through a number of various sources, most of which are veterinary supply stores. These places buy nonsterile straws from IMV (the manufacturer in France) and then package and sterilize the straws for sale. The straws can be sealed with heat or ultrasonically, although we prefer heat to ensure a complete seal.
 - 2) The CBS straws are made of a different plastic that is very cold-stable and much more resistant to damage from cracking and fractures that may occur at low temperatures. CBS straws can be purchased through Irvine Scientific (as of 2013-2014). The basic 0.25cc straw is entitled "embryo straw" and is the same size and shape with a cotton plug at one end, similar to the IMV straws. The CBS straws can be sealed with heat or a specialized SYMS sealer (approx \$2000+). The Syms sealer will provide a very good seal over the years of use, but needs to be checked from time to time for quality assurance. This is the expensive way to ensure, almost idiot-proof, good quality seals.
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How to Heat-Seal Properly



1) To heat-seal, set the control knob to the desired setting (5-7) in most cases; for standard 0.25cc cryo-straws.

You can perform a test on a blank straw by heat-sealing at different settings from 4-8 and check your seals under a microscope to see which setting works best for the straws you are using (See images below).

2) Pre-flattening the ends of the straw is recommended in order to help prevent damage to the edges of the straw while heat-sealing. (see last 2 images)

3) Insert straw between sealer jaws so that the end is sticking out past the heating element. This will ensure a complete, 5mm wide seal.

4) Press and hold the sealer top jaw down firmly. When the red light goes off and the sealer beeps, wait 1-2 sec more before opening the sealer to remove the straw.

5) The straw may stick to the teflon. Do not pull it off as this may disrupt the columns of media and your embryos inside. Gently pry the straw off the teflon using your fingernail. Touching the straw with your fingertip will cool the straw so it can be removed easily. Although the straw will be very warm it should not burn you when you touch it.

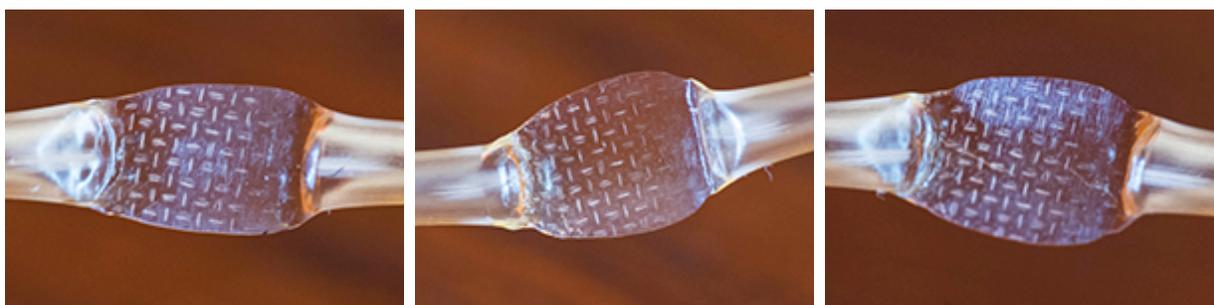
6) IMPORTANT! Check your heat seal under a microscope to ensure proper sealing! See images below.

7) Recalibrate your sealer every 4-6 months to ensure it is working properly.

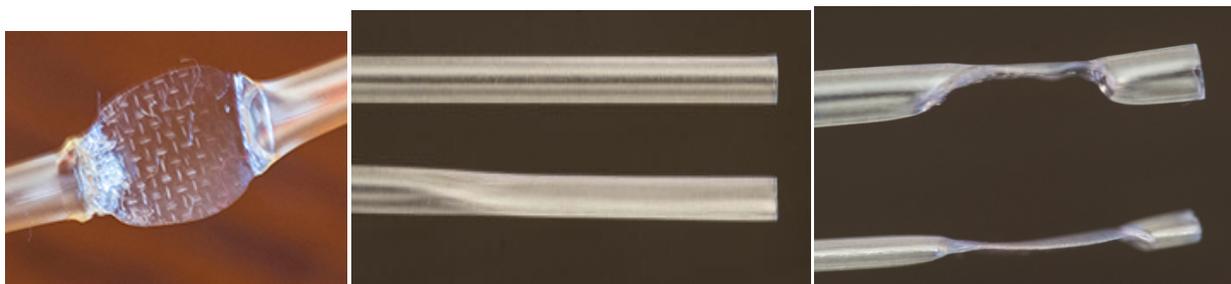
Heat-Seal Images: Heat Settings #2-#8 on AIE-205



Setting #2: Inner lumen showing, not sealed. #3: Inner lumen showing, not sealed. #4: Partial seal, buldge on left side.



Settings #5, 6, 7: Sealed, along length of seal, note how the straw flattens to form a large oval, with marks from teflon. This is a good seal. #6 & #7 are the best, although #5 is good as well.



Setting #8: Sealed, but the straw is a bit damaged on the left edge of the seal because of too high a setting.

The next image is of a regular straw and one that is pre-flattened.

The last image is of the heat seals on the regular vs. pre-flattened straw. Notice how the heat-sealer jaws clamp and push the straw down to seal it. This is much less noticeable in the pre-flattened straw.

- A proper heat seal will melt the straw together.
- No inner lumen should be showing and the straw should flatten out in a large oval shape.
- Check the sides of the straw near the heat seal to ensure there is no damage to the straw at the inner edge of the seal. Notice in the last image that the jaws of the heat-sealer press down to make the seal.
- During sealing, as the straw melts, it will try to regain its shape, which can weaken the straw at the edges of the heat seal. The straw will thin out and may tear at the edge of the seal as it regains its shape. See top straw in last image above.

- Pre-flattening the straw prior to heat sealing will virtually eliminate damage at the edges of the seal.
- Clean the top and bottom jaws periodically with alcohol. Replace teflon if it is damaged. Avoid always sealing in one place along the jaws of the sealer, as this will cause wear or damage in that spot.
- A proper heat seal ensures no liquid nitrogen will enter the straw during storage and prevent the straw from exploding upon warming.