

# IMPULSE HEAT SEALER VALIDATION PROTOCOL

## MATERIALS

- Impulse heat sealer: With heating element width of 2 mm or 5 mm, to seal both ends of the CryoTip®
- 15 CryoTips: Catalog # 40709
- Connector: Catalog # 40736
- 25 µL Hamilton Gastight Luer Tip Syringe or equivalent aspiration tool (using a small volume Hamilton Syringe provides greater control during loading and unloading of the CryoTip)
- Small, sharp surgical grade scissors
- Tweezers or forceps
- Vitrification Solution (VS): Catalog #90132
- Liquid nitrogen (LN<sub>2</sub>)
- Liquid nitrogen reservoir or styrofoam box
- 37°C waterbath
- Minimum 500 mL Beaker
- Thermometer
- Stereo microscope
- Kim wipes

## PROCEDURE

1. Fill the liquid nitrogen reservoir with liquid nitrogen (LN<sub>2</sub>).
2. Attach the syringe to the wide end of the connector and then attach the wide end of the CryoTip to the other (narrow) end of the connector, pull the plunger of the syringe slightly to ensure that there is no capillary action when placing the CryoTip in the VS solution.
3. Slide the metal cover sleeve up along the CryoTip to expose the fine tip end.
4. Load the CryoTip with VS Solution up to the 3rd mark by gently pulling on the plunger of the syringe.
5. Set the impulse sealer to scale #3, holding the CryoTip parallel, place the fine tip on the middle of the heating element and brace your hand to ensure a steady seal. Gently push the handle of the heat sealer down to heat seal the fine tip end at or below the 1st mark. You will hear a beep and the light will turn off to indicate the seal is complete.
6. Observe the seal under the microscope to ensure you have achieved a complete seal. Heat seal the tip one more time just above the first seal, if the seal is not complete.
7. Slide down the cover sleeve to protect the fine tip.
8. Disconnect the CryoTip from the Connector and syringe, set the impulse sealer to scale #6, holding the CryoTip parallel, place the wide end on the middle of the heating element and brace your hand to ensure a steady seal. Gently push the handle of the heat sealer down to heat seal the wide end at or above the 4th mark. You will hear a beep and the light will turn off to indicate the seal is complete.
9. Observe the seal under the microscope to ensure you have achieved a complete seal. Heat seal the tip one more time just below the first seal if the seal is not complete.
10. Plunge the CryoTip (metal covered fine tip side down) into the LN<sub>2</sub> and gently swirl for 3 seconds.
11. Prepare a 500 mL beaker with 37°C waterbath and place the thermometer in the water bath to monitor the temperature.
12. Use the tweezers/forceps to gently grab the wide end of the CryoTip (submerged in LN<sub>2</sub>) and then immediately plunge it into the 37°C waterbath.
13. Wipe the CryoTip dry with a Kimwipe, slide up the metal cover sleeve and carefully observe for leakage of the VS Solution along the entire fine tip end. The same volume of VS Solution (up to the 3rd mark on the CryoTip) should be present if no leakage occurred.
14. Withdraw the plunger of the syringe (with Connector attached) to the half way position.
15. Cut the wide end of the CryoTip on the 4th mark (to remove the heat seal) and then attach it to the Connector and syringe.
16. Apply light pressure to the CryoTip by lightly pushing down on the plunger of the syringe.
17. Carefully observe for leakage of the VS Solution and note the location of any leaks. In rare cases, solution may leak from locations not associated with the heat seal as a result of:
  - exceeding the pressure capacity of the sealed CryoTip
  - cracks in the CryoTip (may occur from damage or compromise of the tip integrity prior to or during use)
  - the heat sealer interface is not sufficiently flexible and may damage the CryoTip with creases or cracks during heat sealing; such heat sealers would FAIL the validation
18. Repeat the entire procedure for a total of 15 CryoTips per validation, to assure consistency (sealer settings and sufficient heat seal) of the heat sealer. Note: Sealer settings may vary between heat sealers, you should adjust your settings accordingly to achieve a complete seal. We suggest writing the sealer settings on the side of the sealer.



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## Acceptance Criteria

### PASS:

When properly heat sealed, there should be no apparent leaks at either heat seal location and no crimp induced cracks on the CryoTips. If a leak occurs at the point of the seal, the heat sealer settings may be too high or too low and should be re-tested at an adjusted scale setting.

### FAIL:

1. Heat sealer is consistently too hot and does not form a secure seal.
2. Heat sealer is not sufficiently flexible at the point of the heating element which could induce crimps and cracks in the CryoTip during sealing.

**NOTE:** If frequent incomplete seal is observed due to wearing off of the heat sealer, the heating element should be replaced and then heat sealer should be re-validated.

The recommended heat settings of the impulse sealer may vary in different heat sealers.

### EXAMPLES OF HEAT SEALED CRYOTIPS:



Example of a good sealed CryoTip



Example of an over-sealed CryoTip



Example of an under-sealed CryoTip



Example of a good sealed CryoTip



Example of an over-sealed CryoTip



Example of an under-sealed CryoTip



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#### IRVINE SCIENTIFIC – CORPORATE

1830 E Warner Avenue  
Santa Ana, CA 92705 USA  
Phone: 1 (949) 261-7800  
Toll Free: 1 (800) 437-5706  
Fax: 1 (949) 261-6522  
Support: [tmrequest@irvinesci.com](mailto:tmrequest@irvinesci.com)