

CryoTip Loading Protocol

ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22–27°C)

As an added precaution during the preparation procedure, carefully examine each CryoTip outside of the package. Prior to use, all CryoTips should be examined under a suitable magnification (40x power) for possible damage (such as tip breakages or cracks) that may have occurred during transport or storage.

Have all necessary materials, tools and equipment ready and easily accessible before starting procedure, including:

- Validated Impulse Heat Sealer (Refer to Heat Sealer Validation Protocol, IS P/N 001429)
- 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)
- Connector: catalog #40736

LOAD, SEAL AND VITRIFY WITHIN SECONDS

(WITHIN 110 SECONDS AFTER INITIAL EXPOSURE TO VITRIFICATION SOLUTION)

- Withdraw the plunger of the 25 µL Hamilton Gastight Luer Tip Syringe or equivalent aspiration tool (with connector attached) to the halfway position.
- Attach CryoTip to connector and syringe (or equivalent aspiration tool).
- Gently slide up metal cover sleeve to expose fine pulled tip of CryoTip.

WHILE VIEWING UNDER MICROSCOPE

1. Aspirate Vitrification Solution (VS) to Mark #1
2. Aspirate specimen(s) and VS to Mark #2

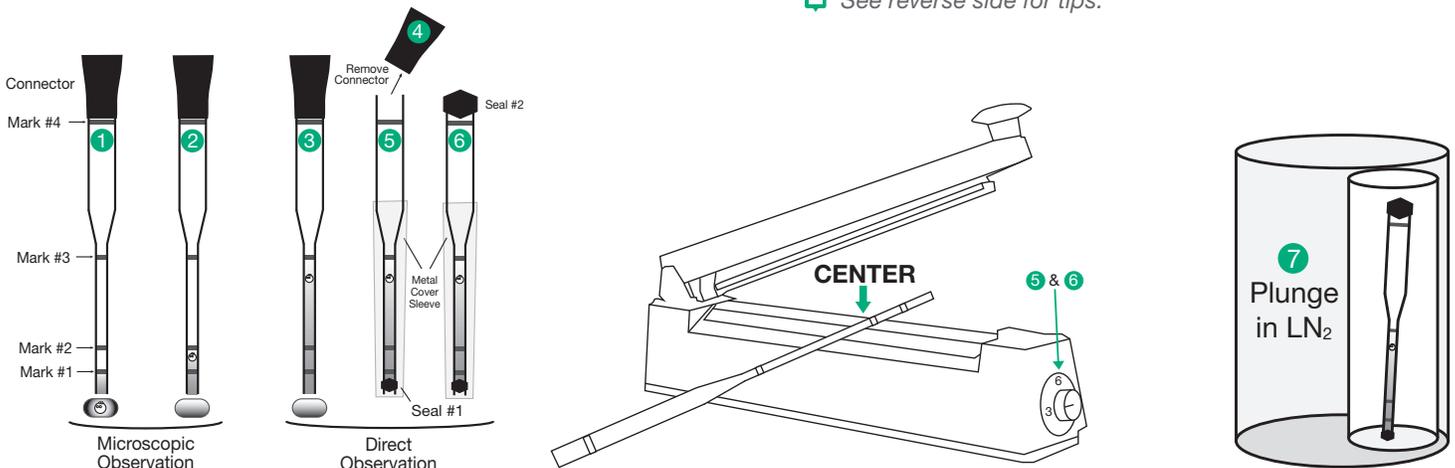
WHILE VIEWING DIRECTLY

3. Aspirate more VS to Mark #3
- ☞ Specimen(s) *MUST* be between Mark #2 and Mark #3.

WHILE VIEWING UNDER MICROSCOPE

4. Disconnect the CryoTip from the Connector and syringe.
5. **Seal #1:** Set the impulse sealer to scale #3, holding the CryoTip parallel to the surface, 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.
 - Slide cover sleeve down to protect tip.
 - Inspect the seal under the microscope to ensure a complete seal.
6. **Seal #2:** 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.
 - Inspect the seal under the microscope to ensure a complete seal.
7. Plunge sealed CryoTip into liquid nitrogen (LN₂) and gently swirl. Ideally, place into an LN₂ filled goblet for long term storage.

☞ See reverse side for tips.



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Tips

- Have all necessary materials, tools and equipment ready and easily accessible before starting procedure (e.g. LN₂ filled holding reservoir, cryocane and goblets, heat sealer, tongs or forceps, pipette or syringe).
- CryoTips should be pre-labeled with patient information, and assembled with Connector and syringe (for loading), prior to starting vitrification procedure. To protect the finely pulled tip from damage, keep it covered with metal cover sleeve until ready to load specimen(s).
- The recommended CryoTip capacity is a MAXIMUM of 2 specimens.
- The timing for exposure to VS is CRITICAL:
 - Load, seal and plunge the CryoTip into LN₂ within 110 seconds after initial exposure to VS drop.
- Rapid and controlled loading of the CryoTip is essential, and requires a secure seal between the CryoTip, Connector and 25 µL Hamilton Syringe or equivalent.
- Carefully attach the connector to the Hamilton Syringe ensuring that there is a secure seal. Next, insert the wide end of the CryoTip gently into the Connector until you have achieved a slight seal.
- The recommended heat settings of the Impulse Sealer may vary in different heat sealers. The appropriate settings for heat sealing both ends of the CryoTip should be determined by following the **Heat Sealer Validation Protocol**, IS P/N 001429.
- CryoTips must remain submerged in LN₂ until ready to thaw. When transferring CryoTips from LN₂ filled holding reservoir, or between LN₂ storage tanks, CryoTips should remain submerged in an LN₂ filled goblet to prevent uncontrolled/premature thawing in air.



Example of a good sealed CryoTip



Example of an over-sealed CryoTip



Example of a under-sealed CryoTip



Example of a good sealed CryoTip



Example of an over-sealed CryoTip



Example of a under-sealed CryoTip

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