CryoJane® User Manual

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(In continental US only)

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(International)

Instrumedics grants the purchaser of the ***CryoJane*** system the rights to use the patented ***CryoJane*** materials

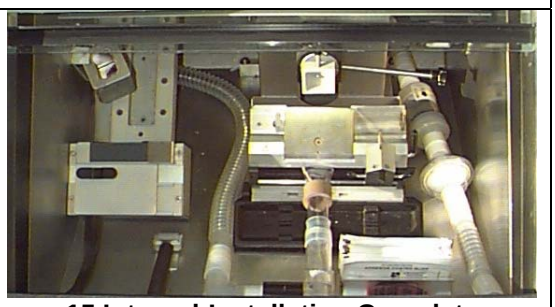
Patent # 5,444,105

CRYOJANE TAPE-TRANSFER
STEP-BY-STEP INSTALLATION INSTRUCTIONS

The following items are required for installation:

Absolute Alcohol **Hair Dryer** **Gauze** **Gloves**

 **01 Remove release paper from ECU**	 **02 Place ECU on top left corner of cryostat**	 **03 Using absolute alcohol and gauze clean chamber wall**
 **04 Heat left wall for Mech/Flash Unit & Oil Bath placement Also heat the exposed adhesive on the mounting plate and oil bath**	 **05 Place Oil Bath towards the rear of the left chamber wall**	 **06 Route Oil Bath & Mech/Flash Unit Cables underneath Mech**
 ***07 Place Mech/Flash Unit in Chamber. Place Mech/Flash Unit low in chamber near the front wall. (Placement near the freeze bar is ideal in most cryostats)**	 **08 Route cables in door bar**	 **09 Place door bar with cables onto front chamber ledge. If required attach Door Bar Support Strip underneath door bar**

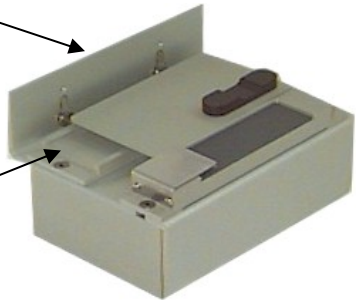
 **10 Adhere Cable holders along cryostat top outer wall**	 **11 ECU Rear with Cable & Power Cord Connectors**	 **12 Connect Mech/Flash Unit, Oil Bath Cables to ECU & Power cord to the ECU and wall outlet Turn the ECU on**
 **13 Mech/Flash Unit Cable connected to ECU**	 **14 External-Installation Complete Cryo-Vac Shown**	 **15 Internal-Installation Complete Cryo-Vac Shown**

***The standard CryoJane Mech/Flash Unit installation is shown in Fig. 07.**

The Microm HM 560 cryostat requires an S-Plate Mech and separate mounting plate for installation.

S-Plate Mech/Flash Unit for the Microm HM 560 Cryostat

Mounting Plate locks onto S-Plate Mech/Flash Unit



Slide Nest Entrance

The entire assembly is adhered to the left cryostat wall as shown in Fig. 07.

Note: **The S-Plate Mech/Flash Unit must have enough clearance from the front wall of the cryostat for slides to enter the Slide Nest.**

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Instrumedics Frozen Section Process Overview

Freezing Process - Conventional Freezing vs. Snap Freezing

In the conventional freezing method, the tissue is typically frozen at about -30°C. During this relatively slow freezing process, the ice crystals that form are usually large compared to cellular dimensions. These crystals can cause considerable displacement and structural damage to the tissue.

The Gentle Jane device is designed to *snap-freeze* the tissue in approximately 8 - 10 seconds. The freezing agent can be liquid nitrogen, LN_2 chilled isopentane, dry ice or a thermoelectric cooler (Peltier device). The temperature at which the tissue is frozen should be - 60°C or colder.

NOTE: Gentle Jane Snap-Freezing methods are described in a separate Snap-Freezing User Manual.

CryoJane Process

The CryoJane process is built around three special methodologies:

1. The capture of an undistorted, thin, snap-frozen section on a special cold adhesive tape.
2. The lamination of the captured section onto a cold glass microscope slide coated with an ultraviolet light curable pressure sensitive adhesive.
3. The curing of the adhesive on the slide with an 8 millisecond ultraviolet flash, and the subsequent removal of the adhesive tape, leaving a still frozen section firmly adhered to the microscope slide.

The CryoJane system is designed to permit the facile application of these methodologies, and can be adapted to virtually all model cryostats.

CryoJane System Components

FIGURE 1. *CryoJane System Components*

1. **Mechanism/Flash Unit. See CRYOJANE MECH/FLASH UNIT COMPONENTS for details.**
2. **Electronics Control Unit (ECU). See CRYOJANE ECU for details.**
3. **½ inch diameter Mech/Flash UnitCable (connected to the Mech)**
4. **Door Channel**
5. **2-Shelf Unit**
6. **Hand Roller**
7. **Adhesive Coated Slides**
8. **Tape Windows**
9. **Knife Facet Wipers**
10. **AC Power Cord (not shown)**

OPTIONAL EQUIPMENT:

11. **Protective Oil Accessories Kit**
    (see **Oil Bath Accessories Kit** sections in this manual)
12. **Cryo-Vac-Away System**
    (see **Cryo-Vac-Away User Manual**)

CryoJane ECU

Figure 2. *CryoJane ECU - back*

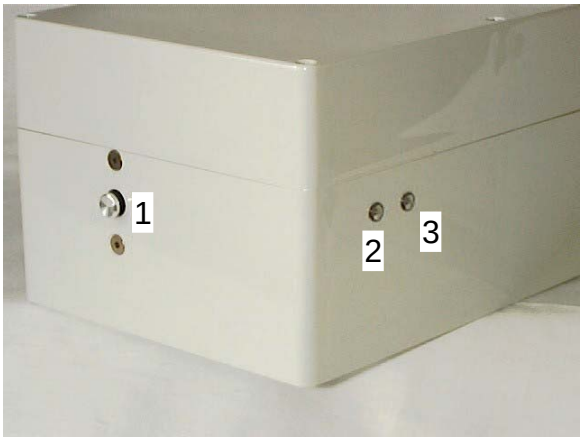

Figure 3. *CryoJane ECU - front and left side*

1. **On/Off Switch**
2. **Pad LED**
3. **Power LED (or Bath LED if optional Oil Bath Accessory Kit is installed).**
4. **Flash/Pad Connector**
5. **Optional Oil Bath Connector**
6. **Fuses**
7. **AC Power Cord Connector**

CryoJane Mech/Flash Unit Components

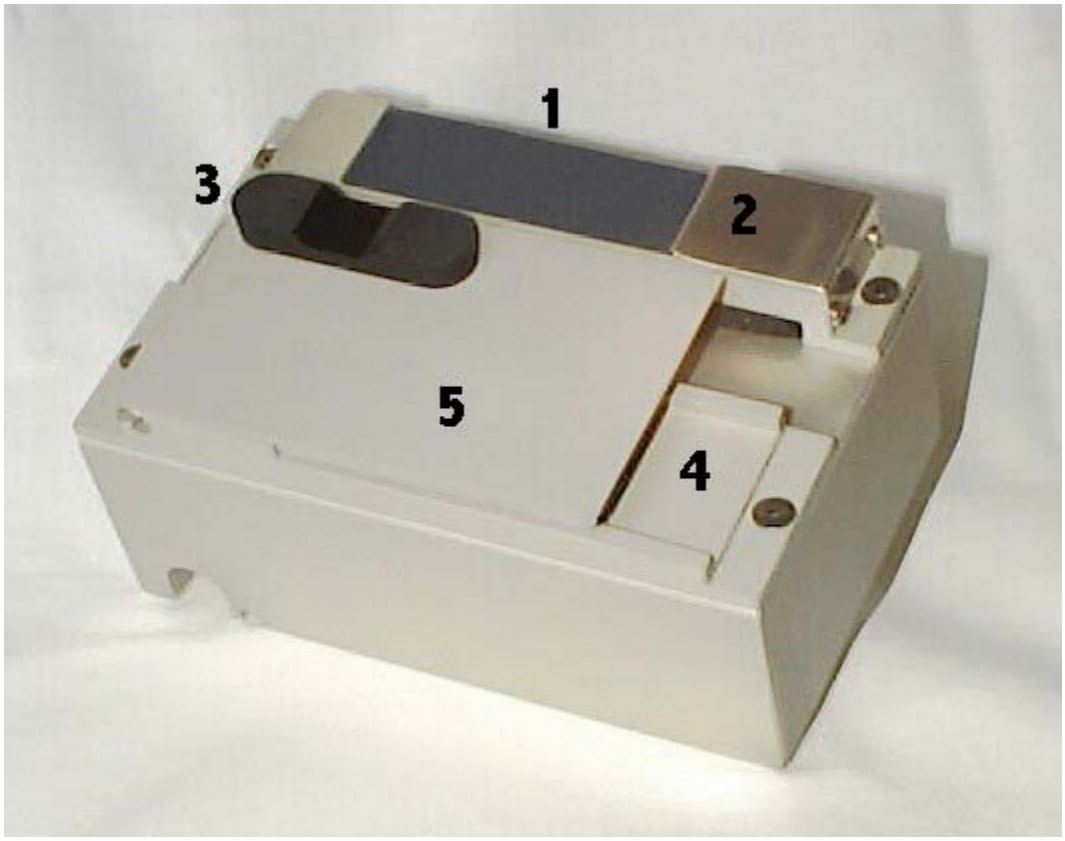

FIGURE 4. *CryoJane Mech Components*

1. **Blue/Gray Pad**
2. **Stainless Steel Spring Clip**
3. **Black Knob**
4. **Flash Tray**
5. **Lid**

CryoJane Operating Procedure

Background

In conventional sectioning the operator usually guides the cut section over the knife using a brush or an anti-roll device. Even with a skillful, steady hand, this step can be difficult, especially if a 2 to 4 micron thick section is desired.

To mount the section, a room-temperature slide is touched to it. When contact is made between the warm slide and the frozen section, instantaneous *melting* of the section occurs, converting the ice crystals back into water. Surface tension causes the melted section to adhere to the slide. When the ice melts, water fills the spaces formerly occupied by the ice crystals, rehydrating the nucleoplasm and cytoplasm, causing erythrocytes to lyse and soluble substances to be displaced. Flow of the melted solutions can distort and displace fine structure. Often the melted section is then dried, during which step, surface tension forces further distort, displace and collapse tissue structure. Pathologists and clinicians have been trained to recognize normal and abnormal structure against this background of degraded fine structure.

In contrast, the CryoJane process is designed specifically to avoid melting and air drying and its associated artifacts. It uses a cold adhesive tape to support and capture the section during the cutting step. The *unthawed* section on the tape is then transferred to a special cold adhesive-coated slide. *The section is not melted in the mounting step.* The adhesive coating on the slide is then polymerized, and the tape is peeled away, leaving the section *permanently* anchored to the slide *while still frozen.*

Preparation

- A day's supply of Adhesive Coated Slides and Tape Windows should be in the cryostat, chilled and ready for use.
- The cryostat temperature should be -25°C to -30°C (depending on the cryostat model).
- The Pad light (left LED) on the ECU should be green.
- The right LED should be red if it is labeled Power and green if it is labeled Oil Bath.

Use

Once a tissue specimen has been frozen and mounted in the microtome chuck, follow the procedure outlined below.

Steps for a Single Section:

1. If the Cryo-Vac-Away is installed, position the collection nozzle at the knife edge and step on the foot switch to automatically remove debris during the trimming sequence. If the Cryo-Vac-Away is not installed, trim the block to the desired depth to obtain a full block face and remove the debris from the knife. Set the micrometer for the desired section thickness and cut two or three sections to be sure a full section will be cut.

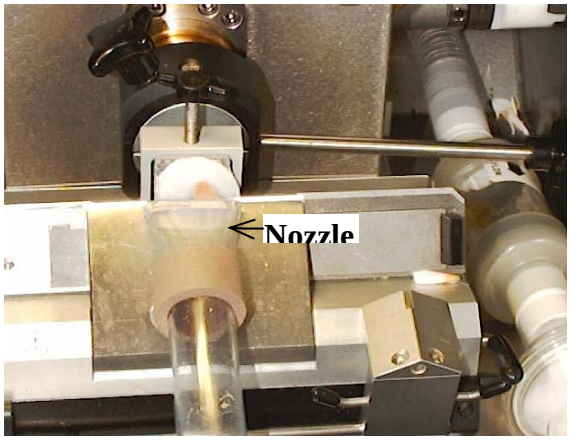

Figure 5. *Block trimming and debris removal.*

2. Take a pre-cooled, pouched, Adhesive Coated Slide, and while holding it **inside** the cryostat chamber, remove the glass slide from the pouch.

Figure 6. *Adhesive coated slide in pouch.*

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3. Holding the Slide at the frosted end, rapidly peel off the protective mylar film which covers the adhesive and discard it.

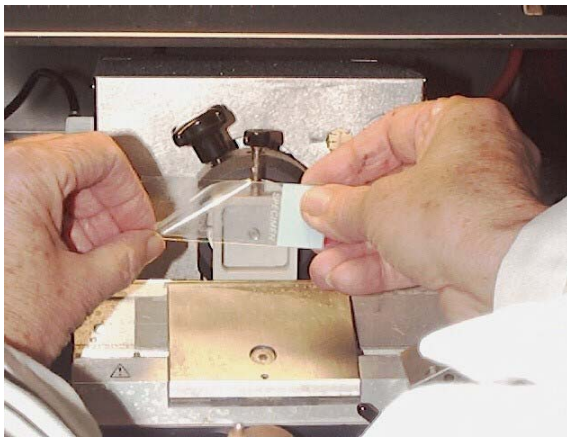


Figure 7. *Peeling mylar from adhesive coated slide.*

4. Place the Coated Slide, adhesive-side-up, on the Blue/Gray Pad of the Mech by slipping the frosted end of the slide under the stainless steel Spring Clip.

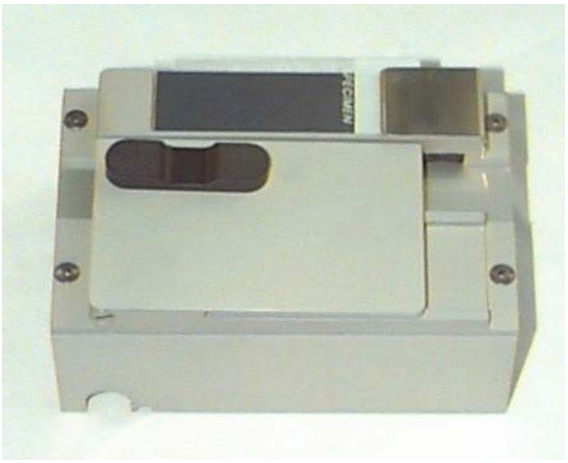


Figure 8. *Adhesive Coated Slide on Blue/Gray Pad.*

5. **Inside** the cryostat, pick up a pink Tape and peel away the protective film covering the adhesive window. Discard the protective film.

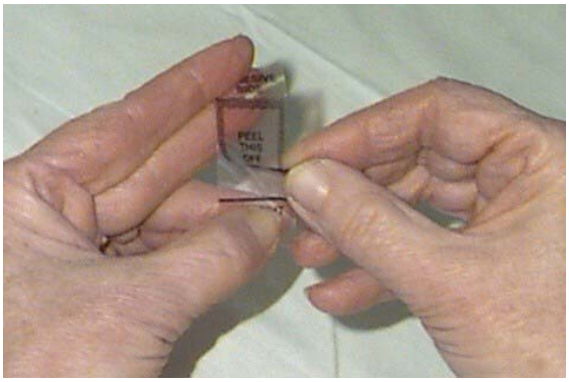


Figure 9. *Exposing the Tape window.*

6. Hold the Tape up by the narrow tab with the adhesive side towards the block face. Position the Tape in front of the trimmed block so that the bottom edge of the exposed adhesive is aligned with the bottom edge of the block. Place the Tape on the block face making sure that the bottom wider tab of the Tape overhangs the knife. ***NO PORTION OF THE ADHESIVE WINDOW SHOULD OVERHANG THE BLOCK FACE.***

   TO AVOID MELTING THE SURFACE OF THE TISSUE DO NOT TOUCH THE PORTION OF THE TAPE THAT COVERS THE BLOCK FACE.

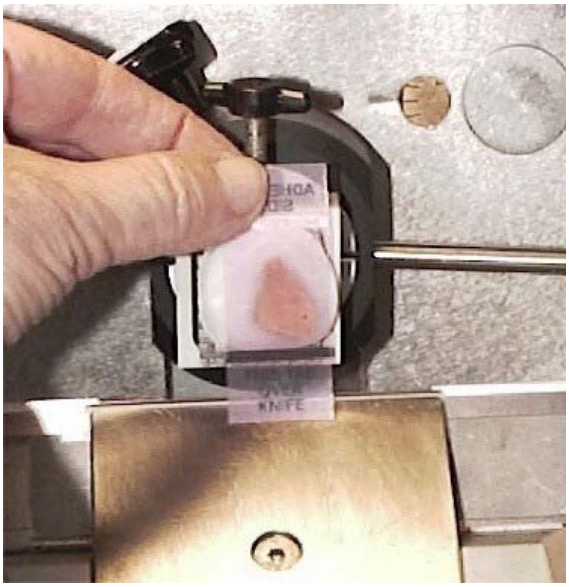


Figure 10. *Placing Tape on block face.*

7. Using the cold Hand Roller, laminate the Tape to the face of the block by moving the Roller up and down one to two times and then left to right one to two times. Some pressure is required for good adhesion. When finished, place the Hand Roller back to its original position **inside** the cryostat.

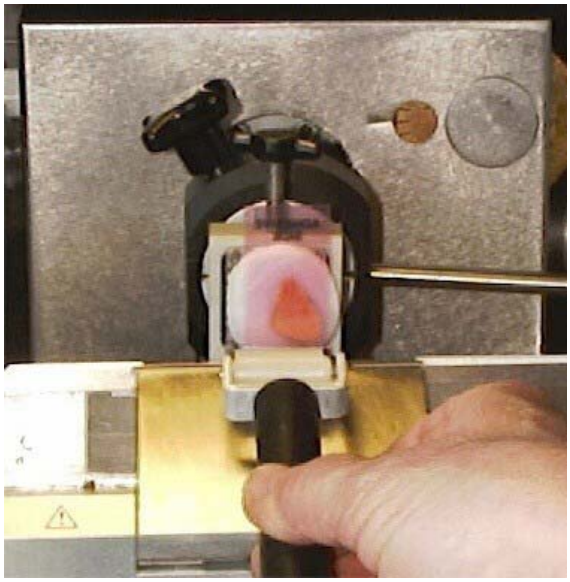


Figure 11. *Laminating the Tape to the block face.*

8. Turn the flywheel slowly and evenly to cut a section. If a ***sharp*** knife is used, the section captured on the Tape will be flat, intact, uncompressed and can be as thin as two microns. ***No need for a brush or an anti-roll device!***

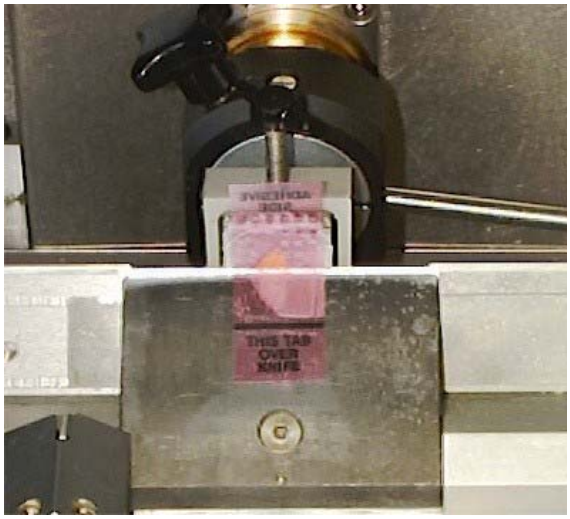


Figure 12. *Cutting the section.*

9. Pick up the Tape at either tab and transfer it to the Slide on the Blue/Gray Pad making sure to keep it down inside the cryostat to avoid melting the section.

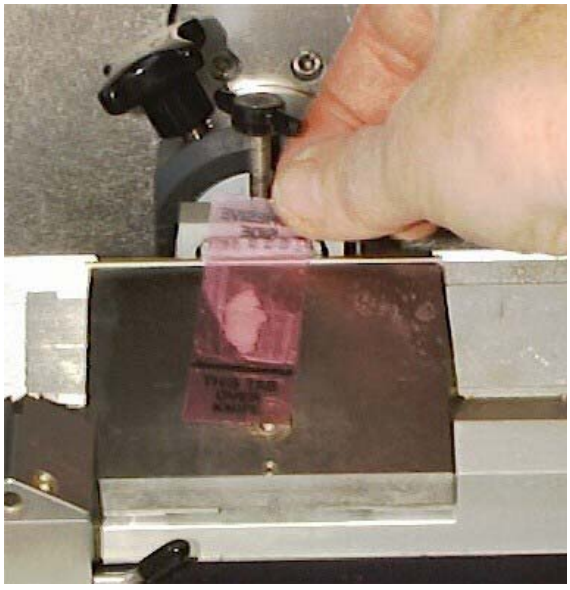


Figure 13. *Handling the cut section.*

10. Position the Tape, section-side-down, on the Adhesive Coated Slide. For multiple sections refer to the **Steps for Multiple Sections**.

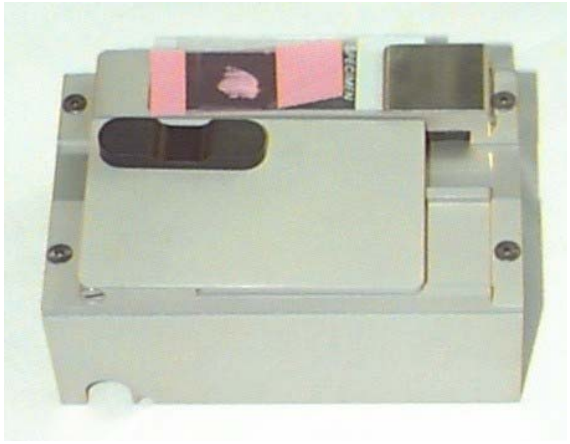


Figure 14. *Positioning the Tape on the Slide.*

11. Use the cold Hand Roller to laminate the Tape to the Slide. Apply even pressure on the Roller and roll it over the section under the Tape two or three times

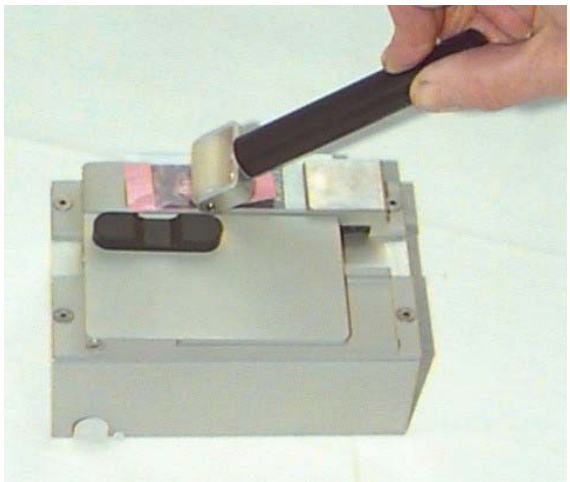


Figure 15. *Laminating the section to the Slide.*

12. Holding the frosted end of the Slide, remove it from the Blue/ Gray Pad and transfer it into the Flash Tray. Gently push the Slide in as far as it will go, against the stop. (To check, lift the Lid.) Close before actuating the flash.

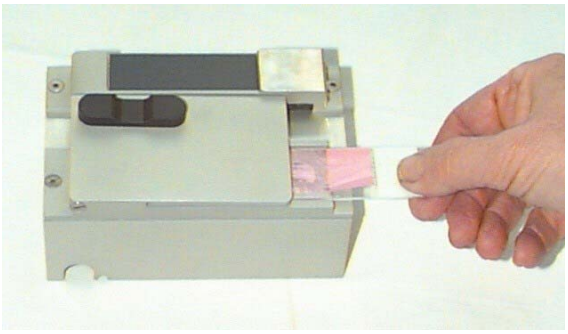


Figure 16*. Placing the slide into the Flash Tray.*

13. Push and release the Black Knob to actuate the UV flash. The UV cures the adhesive on the Slide into a polymer, anchoring the frozen section to the Slide.
    DO NOT FLASH MORE THAT ONCE EVERY 30 SECONDS. MORE OFTEN CAN SERIOUSLY DAMAGE THE ECU**.**

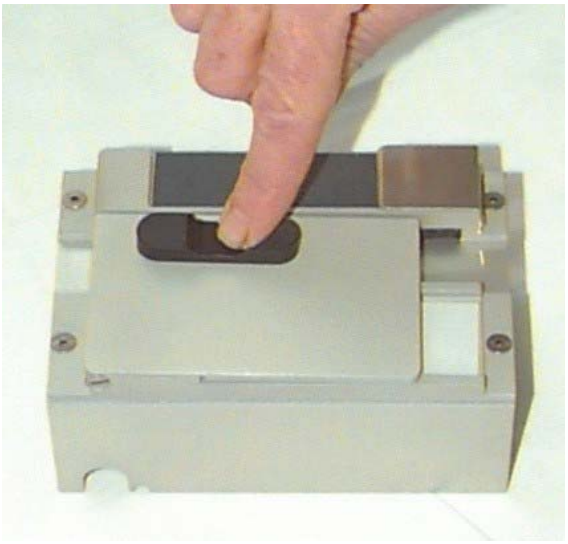


Figure 17*. Curing the adhesive on the Slide.*

14. Holding the frosted end, ***immediately*** remove the cured Slide from the Flash Tray. ***DO NOT TAKE THE SLIDE OUT OF THE CRYOSTAT AT THIS TIME AS THAT WILL CAUSE THE SECTION TO MELT.***

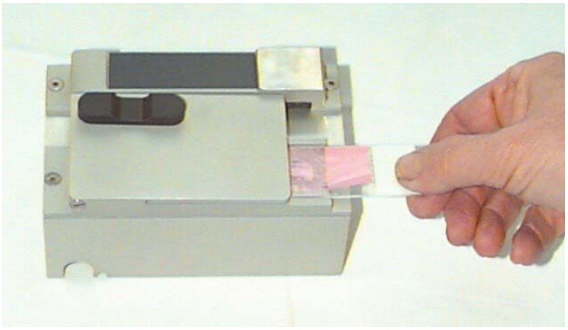


Figure 18*. Removing the cured Slide.*

15. Keeping the Slide ***deep inside*** the cryostat chamber, carefully remove the pink Tape to expose the transferred frozen section on the Slide.

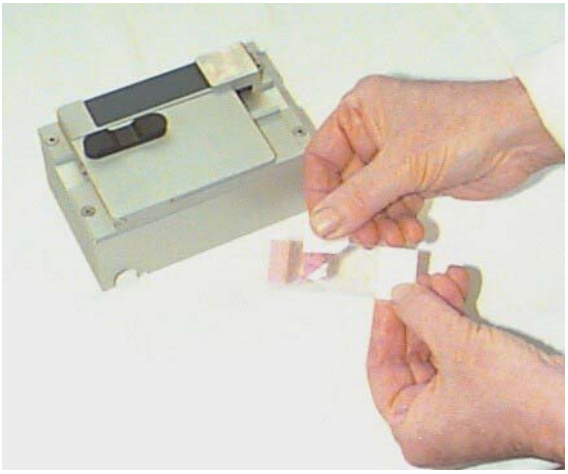


Figure 19. *Removing Tape from cured Slide.*

16. Peel off the Tape diagonally and downward to minimize sheer stress on the section and to insure complete transfer. Do not touch the section with your fingers. Once removed, discard the Tape.

    The slide can now be:
    a) fixed in the ***Instrumedics Aqueous Fixative***,
    b) freeze-dried or freeze-substituted in the cryostat before "anhydrous" fixation,
    c) melted or air-dried and fixed with the fixative of choice for your protocol

    Note: The section should be immersed in the room temperature fixative ***inside*** the cryostat. Fixation should then continue at room temperature.

Figure 20. *Technique for removing Tape from Slide.*

Steps for Multiple Sections:

17. Multiple sections can be positioned on one Slide by placing the Tapes perpendicular to the Slide. For small tissue specimens, the Tape can be cut into narrower strips. Follow **Steps 5 - 9**.

18. To cure the adhesive on the slide, lift the Lid of the Mech and seat the Slide on the Flash Tray. The Tape tabs will overhang the Flash Tray.

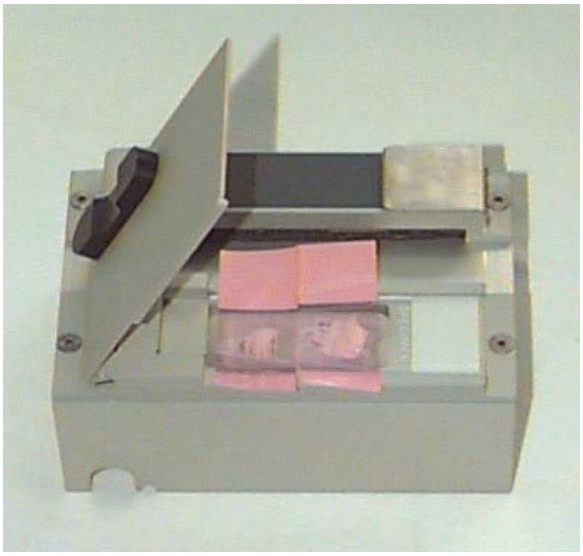


Figure 21. *Processing multiple sections.*

19. Close the Lid and push and release the Black Knob to actuate the UV flash. Then lift the Lid, remove the Slide and carefully peel away each strip of Tape as described in **Steps 15** and **16**.

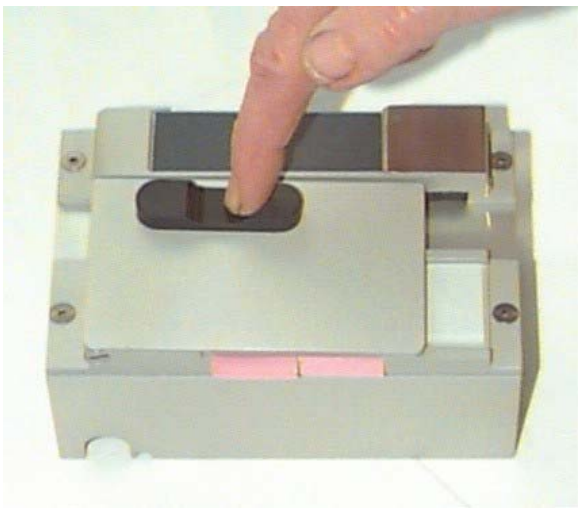


Figure 22. *Curing multiple sections.*

Summary of the Instrumedics Frozen Sectioning Process

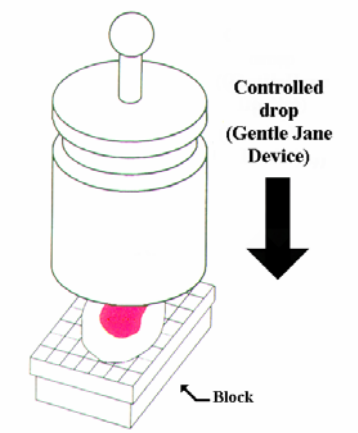


FIGURE 23. *To minimize ice-crystal size, tissue is snap-frozen with a chilled heat extractor.*

Snap-freezing and embedding.

A blockholder is placed on the **Gentle Jane** snap-freezing device. **CryoGel** or other embedding media is dispensed onto the blockholder and a tissue specimen is positioned on top. The chilled heat extractor is placed in its holder and released. When the heat extractor contacts the specimen, the tissue and embedding medium is snap-frozen into a flat block. The plane of the flat block face minimizes trimming. When specimen shape or orientation are essential, the tissue is snap-frozen using the **Instrumedics CryoGel / Rubber Mold** method.

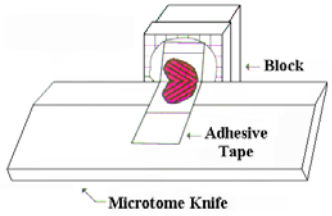


FIGURE 24. *A cold adhesive tape is used to support the section as it is being cut.*

Cutting

After the block is trimmed, a cold adhesive tape is adhered to the block face. The tape supports and captures the section as it is being cut, eliminating the need for a brush or anti-roll device.

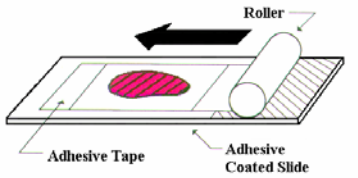


FIGURE 25. *The still frozen section, adhered to the tape is rolled onto the cold adhesive-coated slide.*

Transfer to Slide

A cold adhesive-coated slide is placed on a temperature-controlled pad. The adhesive tape is placed section-side-down on the adhesive-coated slide, and is laminated to the adhesive layer using a cold roller.

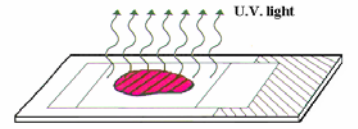


FIGURE 26. *An ultraviolet flash converts the adhesive coating into a hard solvent-resistant plastic.*

Curing the Adhesive Coating

A flash of ultraviolet light passes through the slide to polymerize the adhesive layer on the slide into a hard, solvent-resistant plastic, tightly anchoring the section. to the slide.

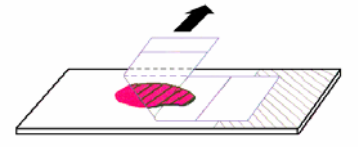


FIGURE 27. *The tape is removed. Several options are available*

Removal of Tape

The tape is peeled away leaving the still frozen section tightly bonded to the plastic layer.

The slide can then be:

a) fixed in the **Instrumedics Aqueous Fixative**,
b) freeze-dried or freeze-substituted in the cryostat before "anhydrous" fixation,
c) melted or air-dried and fixed with the fixative of your choice.

Special Fixation Protocols

***Aqueous Fixative*: THIS FIXATION SHOULD BE USED FOR ALL HISTOLOGICAL STAINING OF CRYOJANE PREPARED SECTIONS.**

This fixative melts and fixes the tissue simultaneously (direct controlled "melting"). Ice crystal artifacts (holes) are masked. Nuclear morphology is usually excellent. Some cell components are lost and most of the red blood cells are lysed.

1. PREPARATION OF AQUEOUS BUFFER
   Dissolve Buffer-Salt Mix in 180 ml of water.
   Buffer-Salt Mix is available from Instrumedics (Cat. #AB)
   Store in refrigerator. Usable for at least 6 months.

2. PREPARATION OF AQUEOUS FIXATIVE
   Add 20 ml of 25% glutaraldehyde to 30 ml of Aqueous Buffer.
   Mix well.
   Use at room temperature.
   Discard after 1 week. (If using 50% glut. add 10 ml glut. and 10 ml water.)

   CAUTION! Contains Cacodylic Acid. Avoid contact with fingers or skin. Do not breathe dust from vial.

3. PROCEDURE
   a) After the adhesive coating on slide has been polymerized, carefully peel off the pink Tape.
   b) To avoid uncontrolled melting and/or drying of the section, immediately bring the room temperature aqueous fixative into the cryostat and immerse the slide into the fixative. Dip 2-3 times.
   c) Continue to fix for 15 to 30 seconds at room temperature.
   d) Rinse the slide in water and proceed with a chosen staining protocol.

"Anhydrous" Fixation Protocol - To be used after "freeze-substitution" in a cold solvent such as acetone at -30°C or colder.

"Anhydrous" fixation preserves *most* cellular components and is highly recommended for routine stains. Note: Not recommended when ice crystal size is large and widespread (poor freezing). In such cases the aqueous fixative protocol should be followed. An "anhydrous" fixative contains a maximum of 30% water.

1. AN/DMF/Glut

Dimethylformamide (DMF)	35 ml
Acetonitrile (AN)	35 ml (can use 70 ml DMF and no AN)
glutaraldehyde	10 ml 25% Glut (5 ml 50% Glut)
Add H_2O to make up to	100 ml
Wait at least 1 our before use.	

2. **Formal-Ethanol**

Ethanol (100% alc.)	86 ml
Formaldehyde	14 ml

Bring the fixative into the cryostat and transfer the freeze-substituted slide directly from the acetone bath into the fixative. Continue fixation at room temperature for 15 to 30 seconds. (Transfer is made *within* the cryostat to prevent condensation of water on the cold slide prior to fixation.)

3. ***For very sensitive antigens: Fix in the cold for 1 min.***

DMF/Glut

Dimethylformamide	70 ml
25% Glutaraldehyde	1 ml
Add H_2O to make up to	100 ml

Transfer the freeze-substituted slide directly from acetone to the fixative at -4 C for 30 to 60 seconds. This transfer must be made *within* the cryostat.

Following fixation, the slides are rinsed in water prior to IHC protocols

REFER TO REAGENT MSDS FOR SAFE HANDLING

CryoJane Maintenance

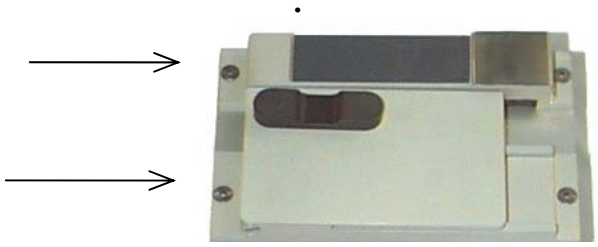
ECU

- NOTHING SHOULD BE PLACED OR STORED ON TOP OF THE ECU! Any spills which enter the ECU may severely damage the electronic components.
- To clean the ECU, wipe the surfaces with either a dry rag, or if necessary, an alcohol wipe. ACETONE OR OTHER SOLVENTS WILL DAMAGE THE PLASTIC SURFACES.
- Fuses can be replaced by unscrewing the two small fuse holders in the rear of the ECU. Fuses should be **0.75 AMP SLO-BLO** , (available from Instrumedics).

DO NOT OPEN THE ECU! Only personnel authorized by Instrumedics should service the ECU. Unauthorized opening of the ECU can cause serious injury and will void all warranties. Unauthorized persons will assume all risks and responsibilities.

Mech/Flash Unit

- All metal surfaces may be wiped with alcohol or chlorine solution.
- The Flash Tray should periodically be cleaned with alcohol to remove any adhesive build-up.
- Care must be taken to avoid damaging the glass filter in the Flash Tray.
- Liquid spills on the Mech/FlashUnit should be wiped immediately. Electronic components in the Flash Lamp may short circuit if exposed to liquids.
- DO NOT IMMERSE THE MECH/FLASH UNIT IN ANY SOLUTIONS.
- To remove the Mech/Flash Unit from the wall for decontamination or servicing, simply unscrew the two screws shown below and then remove the Mech from the cryostat. The Mech/FlashUnit's mounting plate will remain attached to the wall of the cryostat. (Tip: To avoid losing the two screws, screw them into the holes in the mounting plate remaining on the wall, attach it to the mounting plate with the two screws.



Troubleshooting the Mech and ECU

- ***The LEDs (lights) on the ECU are not on.***

Cause	*Solution*
The ECU is not turned on.	Make sure the ECU is properly connected to an electrical source. Make sure the On/Off Switch on the ECU is in the On position (pushed in). If the LEDs are on that indicates that the ECU has power.
One or both fuses in the back of the ECU are blown.	Check the fuses on the back of the ECU and replace as needed. *Use only 0.75 AMP, SLO-BLO fuses (available from Instrumedics).*
The ECU is malfunctioning.	Call Instrumedics' customer service number and arrange for servicing of the ECU. DO NOT OPEN THE ECU!

- ***The left LED (labeled Pad) always displays a solid red light.***

Cause	*Solution*
The ½ inch diameter Flash/Pad Power Cable is not connected properly.	Make sure the Flash/Pad Power Cable is properly connected to the ECU and to the Blue Pad connector underneath the Mech.
The temperature of the Blue/Gray Pad is too cold.	Raise the cryostat temperature.
The ECU is malfunctioning.	Call Instrumedics' customer service number and arrange for servicing of the ECU. DO NOT OPEN THE ECU!

- ***The left LED (labeled Pad) always displays a flashing red light.***

Cause	*Solution*
The temperature of the Blue/Gray Pad is too warm. If the sections are not melting on the pad ignore the flashing red light	Lower the cryostat temperature.
The ECU is malfunctioning.	Call Instrumedics' customer service. I **DO NOT REMOVE THE LID OF ECU! THE ECU LID SHOULD ONLY BE**

	REMOVED BY AUTHORIZED PERSONS

ONLY IF THE OIL BATH OPTION IS INSTALLED:

- ***The right LED (labeled Oil Bath) is always red.***

Refer to the **Oil Bath Accessory Kit Troubleshooting Guide** in this manual.

- ***The Flash does not go on when triggered.***

Cause	***Solution***
The ECU is not turned on.	Make sure the ECU is properly connected to an electrical source. Make sure the On/Off Switch on the ECU is in the On position (pushed in). If the LED's on the front of the ECU are on, that indicates that the ECU is on.
The ½ inch diameter Flash/Pad Power Cable is not connected properly.	Make sure the Flash/Pad Power Cable is properly connected to the matching connector on the rear of the ECU.
The fuses may be blown One or both fuses in the back of the ECU are blown.	Check the fuses on the back of the ECU and replace as needed. ***NOTE****: If flash unit is triggered more than 2 times in one minute, one or both fuses on the back of the ECU will blow out and will need to be replaced. This is a protective feature designed to prevent overheating. Use only* ***0.75 AMP, SLO-BLO****, Ceramic Core Wound fuses (available from Instrumedics).*
The Flash triggering mechanism is jammed.	Check for ice build-up around the Black Knob on the Mech and remove if found. If this problem cannot be corrected, call Instrumedics' customer service number and arrange for servicing of the triggering
The flash unit is malfunctioning.	Call Instrumedics' customer service number and arrange for servicing of the flash unit.. **DO NOT OPEN THE FLASH UNIT!**
The ECU is malfunctioning.	Call Instrumedics' customer service number and arrange for servicing of the ECU. **DO NOT OPEN THE ECU**

CryoJane Process Troubleshooting

The CryoJane process will not produce the desired results if the cryostat and/or the microtome are malfunctioning. Both need to be serviced periodically. Check with the manufacturer/dealer.

♦ *The Tape does not adhere to the block face.*

Cause	*Solution*
The protective film covering the adhesive is not removed.	Peel away the protective film covering the adhesive window which says "PEEL THIS AWAY"
The block is too cold or too warm.	Check the temperature of the cryostat and adjust if necessary. Chamber temperature should generally be set between -25°C to -27°C depending on the particular cryostat model.
There is moisture on the adhesive layer of the tape or on the blockface The tape will not adhere to the blockface if there is condensation on either the tape or the blockface.	Check to see if the cryostat needs to be defrosted. In warm, humid weather keep the cryostat lid closed as much as possible The tape will not adhere to the blockface if there is condensation on either the tape or the blockface.

♦ *The Tape does not capture the section.*

Cause	*Solution*
The microtome is not advancing.	Check the microtome and adjust.
The microtome is not advancing at the desired thickness.	Check the microtome and adjust. Cut two or three sections before applying the Tape.
The knife angle is too small or too large.	Adjust the knife angle between 3 and 5 degrees.
The Tape is defective.	Use another Tape.
The Tape does not stay adhered when the section is being cut.	The Tape is overhanging the blockface and snagging on the knife which causes the tape to pull away. Cut the tape so that it fits the block.
The blockface is not flat.	The block should be faced off (trimmed) so the the blockface is flat. The Tape should be adhered to the entire surface.

♦ *The Tape is cut during sectioning.*

Cause	*Solution*
The block face is not flat.	Align and trim the block to obtain a flat face.
The adhesive on the Tape overhangs the block and snags on the knife. Cut the Tape so that is fits the blockface.	Position the Tape on the block so that ***no*** adhesive is exposed to the knife when cutting the section.
The knife and/or the block are not be properly	Check the knife and block for looseness and

tightened.	tighten all screws and clamps as needed.

♦*The captured section on the Tape is not intact.*

Cause	*Solution*
The most common cause is the section is not laminated with enough pressure to get good adherence to the adhesive layer.	Use pressure when you roll over the section to laminate to the adhesive layer on the slide. If you are using CJ-1X slides you may want to try CJ-4X
The knife is dull.	If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. If using a stainless steel or tungsten-carbide knife, sharpen the knife.
There is dirt on the Tape	Use a fresh, clean and cold Tape.
Bubbles form while laminating the Tape to the block face.	Apply the Tape "like wallpaper" to the block face while laminating with the cold Roller. If bubbles are still visible, reapply the Tape.
There is vibration in the microtome (thick/thin, chatter, etc.)	Check the knife and block for looseness and tighten all screws and clamps as needed.

♦*The Transferred Section is not Intact. Parts of the Section Remain on the Tape.*

Cause	*Solution*
The section does not transfer intact	Remove the Tape slowly and carefully, peeling it diagonally and downward to minimize the tension on the section.
The knife is dull.	If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. If using a stainless steel or tungsten knife, sharpen the knife.
The Tape was not well adhered to the adhesive layer on the slide in the laminating step.	Pressure must be exerted when rolling the tape with the section on to the adhesive layer on the slide to insure the section is well adhered. This is critical for good transfer.
A bubble may have formed when the Tape was laminated to the Slide.	Apply the Tape "like wallpaper" to the Slide while laminating with the cold Roller.

Troubleshooting Finished Slides

- ***The polymerized adhesive picks up excessive red stain after H&E.***

Cause	*Solution*
Eosin may be too acidic or too alcoholic. This may cause background staining.	Stain a shorter time, or use an aqueous eosin Y at pH 5.0 - 5.2.

- ***There are chatter marks ("venetian-blind" effect, parallel to knife edge) in the section.***

Cause	*Solution*
There is looseness in the system	Check tightness of chuck. Clamp knife holder and/or blade holder very tightly.
The knife is dull.	If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. If using a stainless steel or tungsten knife, sharpen the knife.
Stop/start motion during cutting.	Cut slowly without stopping. If motor drive is used, set cutting speed slower.

- ***There are tears in the section.***

Cause	*Solution*
The knife has a nick or defect on the cutting edge.	If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. If using a stainless steel or tungsten knife, sharpen the knife.

- ***Small clusters of cells (usually circular) are missing in the section.***

Cause	*Solution*
Bubbles or debris was captured between the adhesive Tape and the block face, or between the Tape and the coated Slide.	To avoid bubbles, apply Tape "like wallpaper". If bubbles are visible under the Tape on the block face, reapply the Tape. Use clean Tape.
A hole in the block face may be due to groups of cells being "plucked-out" during or prior to sectioning.	This may be a defect in the tissue block. Check the knife edge for dullness and correct.
Debris was caught between the Tape and block, or between the Tape and the coated Slide.	Always remove all trimming debris from the block face, and both the front and rear surfaces of the knife's cutting edge before sectioning.

- ***There are holes in the nuclei and/or cytoplasm of the section.***

Cause	***Solution***
The freezing process is slow and the temperature in not low enough to prevent damaging large ice crystals from forming. Section may have melted and refrozen causing large ice crystals to grow. (This is the most common problem.)	Snap-freeze tissue at -60°C or colder. Using Instrumedics' ***Stand Alone Gentle Jane*** snap-freezer with liquid nitrogen produces the best results. Snap-freeze the thinnest practical specimen. The sections uppermost in the block will have the minimum ice crystal artifact. Transfer the frozen block from the freezing device ***rapidly*** to the cryostat to avoid thawing the tissue. Avoid finger contact with the central portion of the Tape and/or Slide. Do not touch the block face with your fingers (thumb). Do not breath directly on the section. Keep Tapes and Slides inside the cryostat. The Blue/Gray Pad temperature may be too warm - See the **Mech and ECU Troubleshooting Guide.** ***ALL STEPS OF THE CryoJane PROCESS MUST BE CARRIED OUT DEEP INSIDE THE CRYOSTAT CHAMBER TO AVOID WARM AIR CONTACTING AND MELTING THE FROZEN SECTION.***
	Use Instrumedics' Aqueous Fixative. NOTE: Excellent results with freeze-substitution followed by "anhydrous" fixation will occur *only* if the ice crystal size is minimal.

Oil Bath Accessories Kit

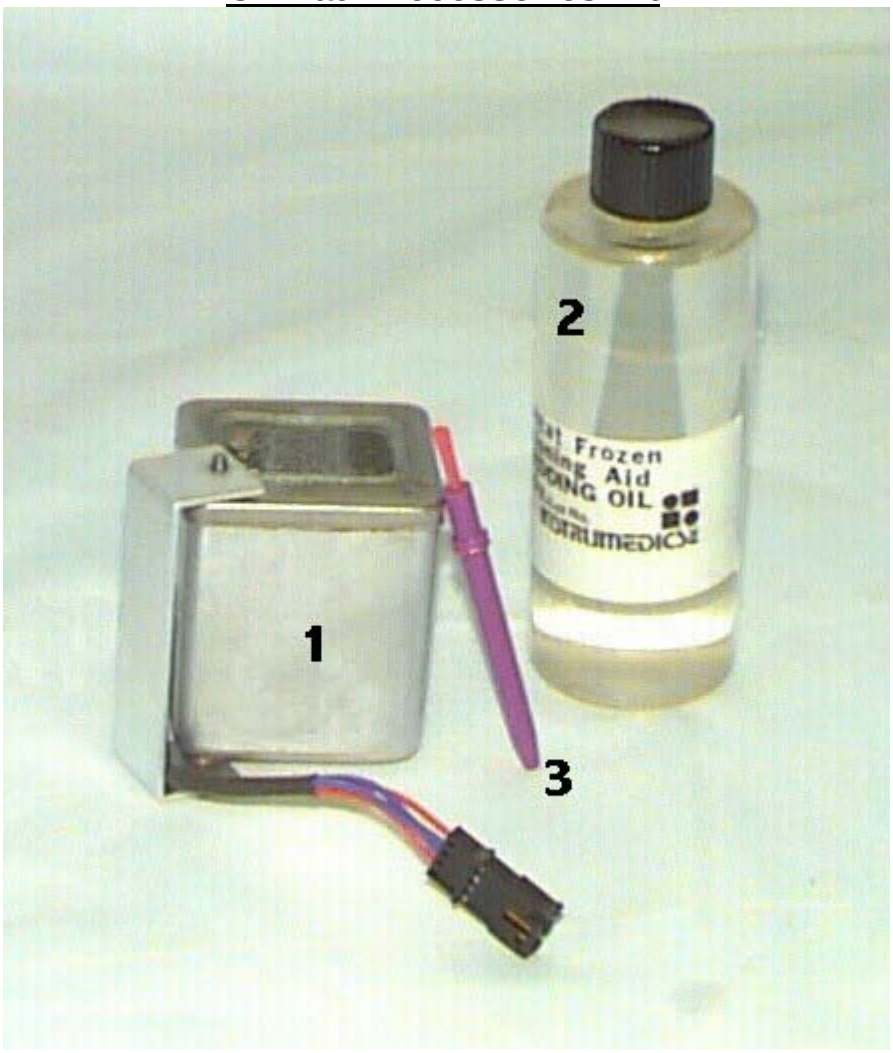


FIGURE 28. *Oil Bath Accessory Kit Components*

1. **Oil Bath**
2. **Protective Oil®**
3. **Oil Brush**
4. **Oil Bath Power Cable (not shown)**

Oil Bath Accessory Kit Operating Procedure

Background

Virtually all embedding media are water based. When a frozen tissue specimen embedded in such media is stored, over time the tissue and the medium will dehydrate and degrade. The Oil Bath Accessory Kit enables the user to coat the block face with the Protective Oil that is maintained at -8°C in a bath inside the cryostat chamber. Coating the surface will not melt the tissue in the block. The Protective Oil has a freezing temperature of -10°C and will instantly freeze on the block face. The frozen Oil coating prevents tissue and medium dehydration for up to one year if stored in a -70°C freezer.

The Protective Oil can also be used to repair a damaged block. A deformation or hole in the block face may be filled with the Oil, which will freeze. The frozen Oil has the property of an embedding medium and can then be sectioned similarly to other media.

Preparation

- The cryostat temperature should be -25°C to -30°C.
- Make sure there is Oil in the Bath. If Oil is added to the Bath, wait 1 to 2 hours for the Oil to reach its set temperature of -8°C.
- The Protective Oil inside the Oil Bath should be in a liquid state (not frozen).
- The Oil Brush should be inside the Oil Bath.
- The right LED on the ECU (labeled Oil Bath) should be green.

Use

A tissue block can be stored in a freezer at -80°C for up to 1 year once thoroughly coated with the Protective Oil.

THE BLOCK MUST NOT BE PERMITTED TO THAW AND REFREEZE DURING STORAGE. THIS WILL DEGRADE THE QUALITY OF THE TISSUE IN THE BLOCK

Storing a Tissue Block

To store (short term in the cryostat or long term in a freezer) a frozen tissue block follow the procedure outlined below.

For Short Term Storage in the Cryostat

With the block in the cryostat, use the Oil Brush to coat the block face with the Oil from the Oil Bath.

For Long Term Storage in a -80°C Freezer

With the block in the cryostat, use the Oil Brush to coat the *entire* block with the Protective Oil from the Oil Bath. Apply generous amounts of Oil.

Figure 29. *Coating the block with Oil.*

Sectioning a Stored Tissue Block

If the block has been stored in a freezer at –80C place the block in the cryostat for at least an hour before sectioning.

Trim away the frozen layer of Oil to expose the tissue specimen and proceed as usual.

Oil Bath Accessory Kit Troubleshooting Guide

- ***The Oil inside the Oil Bath is frozen.***

Cause	*Solution*
The ECU is not turned on.	Make sure the ECU is properly connected to an electrical source. Make sure the On/Off Switch on the ECU is in the On position (pushed in). If the LED's on the front of the ECU are on, that indicates that the ECU is on.
The Oil Bath Power Cord is not connected properly.	Make sure the Oil Bath Power Cord is properly connected to the ECU and the Oil Bath.
One or both fuses on the back of the ECU are blown.	Check the fuses on the back of the ECU and replace as needed. *Use only 0.75 AMP, SLO-BLO fuses (available from Instrumedics).*
The cryostat temperature is too cold.	Raise the cryostat temperature.
The ECU is malfunctioning.	Call Instrumedics customer service number and arrange for servicing of the ECU **DO NOT OPEN THE ECU!**

- ***The Oil Bath LED always displays a solid red light.***

Cause	*Solution*
The Oil Bath Power Cord is not connected properly.	Make sure the Oil Bath Power Cord is properly connected to the ECU and the Oil Bath.
The temperature of the Oil inside the Oil Bath is too cold.	Raise the cryostat temperature.
The ECU is malfunctioning.	Call Instrumedics' customer service and arrange for servicing **DO NOT OPEN THE ECU!**

- ***The Oil Bath LED always displays a flashing red light.***

Cause	***Solution***
The temperature of the Oil inside the Oil bath is too warm.	Lower the cryostat temperature.
The ECU is malfunctioning.	Call Instrumedics customer service and arrange for servicing of the ECU. **DO NOT OPEN THE ECU!**