

EMS-002 Cryo Workstation

Accessories



2. Cryo-TEM Transfer Interface

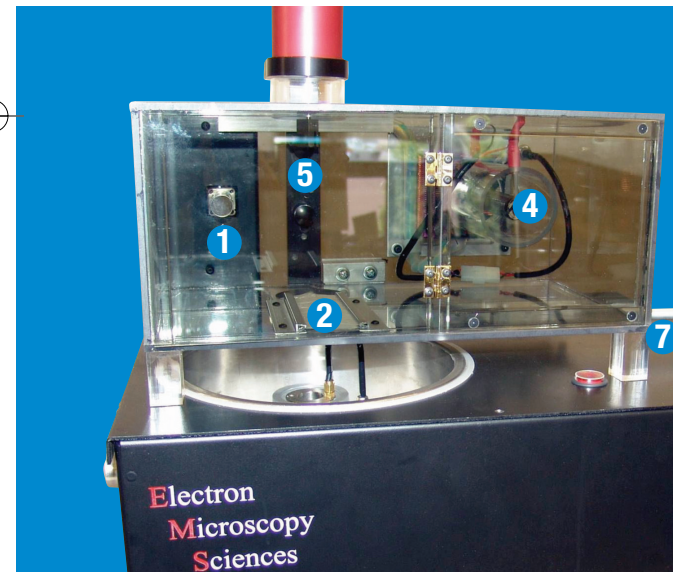
It is possible to directly transfer your vitrified samples using the Cryo-TEM transfer interface, along with your Gatan 3500 series ultra-low temperature, tilt rotate specimen holders. The transfer interface allows the direct insertion of Gatan cryo-holder into the plunge freezer's liquid nitrogen dewar. This will allow easy cryogenic transfer of the vitrified sample directly to the microscope via the 3500 cryo-holder right from the work platform within the dewar. Please consult the EMS product manager for options as the transfer holder is not compatible with all possible plunge freezer configurations.
Part # 37000-T

3. Environmental Chamber

Some plunge freezing applications will require a controlled environment for the samples to be frozen in a satisfactory manner. The environmental chamber module can provide specific relative humidity and temperatures required for many types of samples. The dry nitrogen atmosphere below the environmental chamber allows us to precisely adjust the relative humidity, upwards, and easier to add water in this case. We choose to control the temperature then manually adjust the humidity by adding small quantities of water to the environment by vaporization. The vaporized water is quickly and evenly distributed throughout the chamber by a circulation fan. The integrated shutter mechanism prevents the sample from precooling before it is rapidly frozen.

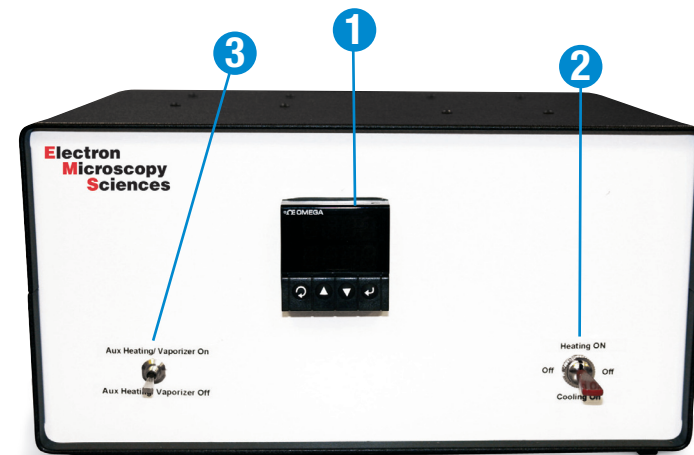
Instrument Overview

- 1. Temperature & Humidity Sensor:** Sends temperature & humidity data to controller.
- 2. Baffle Comb:** Allows used to transfer frozen sample to be transferred to the plunge freezer's "Work Platform" without allowing the Dewar gas to enter the chamber while shutter is closed.
- 3. Shutter:** Keeps the controlled atmosphere inside the Chamber till the sample is to be frozen. It also can prevent pre-cooling of the sample. (Not shown)
- 4. Heating & Vaporizer:** This provides a good portion of the heat load. It also serves to vaporize added water if the humidity is too low.
- 5. Vertical Mounting Bar:** Serves to anchor the Chamber to the Cryo Workstation.
- 6. TEC (Thermo Electric Cooler):** This assembly has a solid state device at its center that heats & cools depending on the polarity of the voltage applied. Used for addition heating and cooling but its main function is to lower humidity if the humidity is too high.
- 7. Shutter Re-set:** Plunger is pressed in firmly to lock the shutter in the closed position.
- 8. Water Tube:** Is used to introduce tiny amounts of water into the Heating/Vaporizer assembly if the humidity inside the chamber is too low. (Not shown)



Controller Front Overview

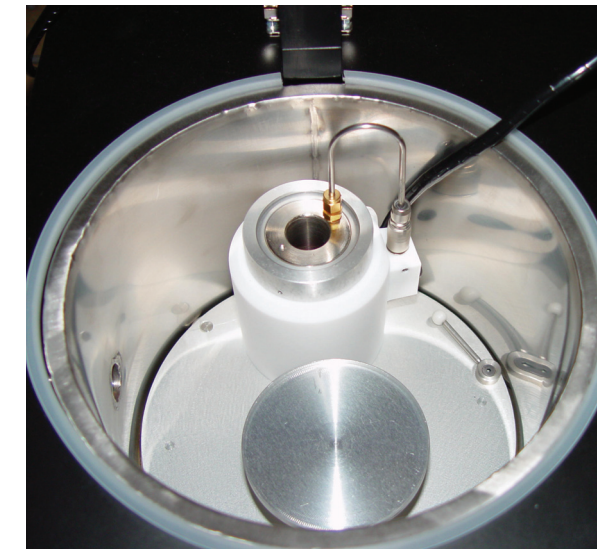
- 1. Controller:** Used to maintain temperature inside of the chamber and MONITOR Humidity only.
- 2. Heat/Cool Selector Switch:** Allows user to select additional heating for the chamber or cooling which can be used to reduce the humidity in the chamber.
- 3. Aux Heating/Vaporizer Mode Switch:** Simple on-off switch to turn on the vaporizing chamber, rapidly adding water vapor to the environment.



Part # 37457

Accessories (continued)

4. Metal Mirror Freezing Kit



This technique has been widely used for fast freezing of very small samples where you only need 5 to 10um of good freezing while maintaining specimen orientation. Metal mirror freezing has been available for quite some time and was used extensively in conjunction with freeze fracture, and deep etch rotary shadow. In the traditional method, the sample was placed on top of a slice of aldehyde fixed lung hung upside down. Once mounted its accelerated onto the surface of a copper block that was cool to liquid nitrogen temperature. Some investigators even used liquid helium to achieve higher rates of thermal transfer between the copper block and sample.

Part # 37455

5. Diamond Anvil

Special Freeze Anvil with real diamond plate for better rapid freezing and easier specimen removal. Does not require polishing like standard Copper anvils.

Part # 37015

6. Automatic Liquid Nitrogen Filling

Keeps constant LN₂ level in the Dewar Automatically. (NOTE: To be used only in conjunction with basic plunge freezing or freezing substitution.)

Part # 37454

7. Propane Transfer System

Allows for the efficient transfer of propane to the condensation chamber from the propane storage vessel.

Part # 37015

8. Freeze Forceps

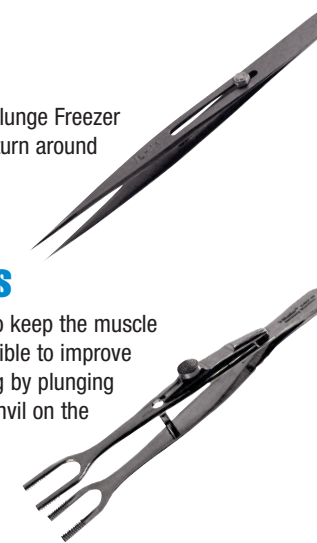
Special tweezers specifically for use with the Plunge Freezer that is "self-locking". Extra sets allow quicker turn around when freezing multiple samples.

Part # 37458

9. Muscle Biopsy Clamps

Clamps designed for human Muscle biopsies to keep the muscle from contracting during fixation. Now it is possible to improve the morphology of the sample by rapid freezing by plunging into liquid cryogen. Fits directly into the drop anvil on the Plunge Freezer.

Part # 37459



Ordering Information

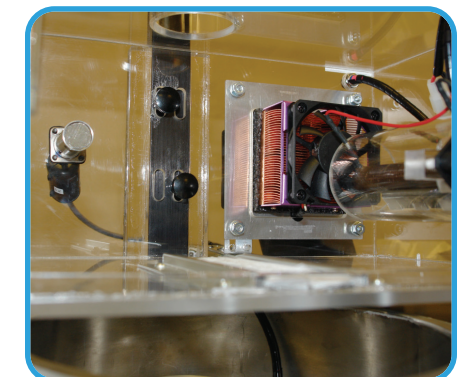
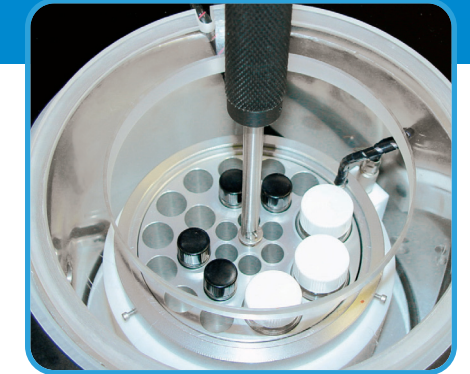
37000	EMS-002 Cryo Workstation Complete with Removable Drop Anvil Assembly, Stainless Steel Liquid Nitrogen Dewar, Cryogen Temperature Controller, Cryogen Holder, and Instructions. 110V-240V	37457	Environmental Chamber
34765	Freeze Substitution Module	37455	Metal Mirror Kit
37000-T	Cryo Transfer Face for TEM	37456	Diamond Anvil
		37454	Automatic Liquid Nitrogen Filler
		37015	Propane Transfer System
		37458	Freeze Forceps
		37459	Muscle Biopsy Clamps

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EMS-002 Cryo Workstation

Overview

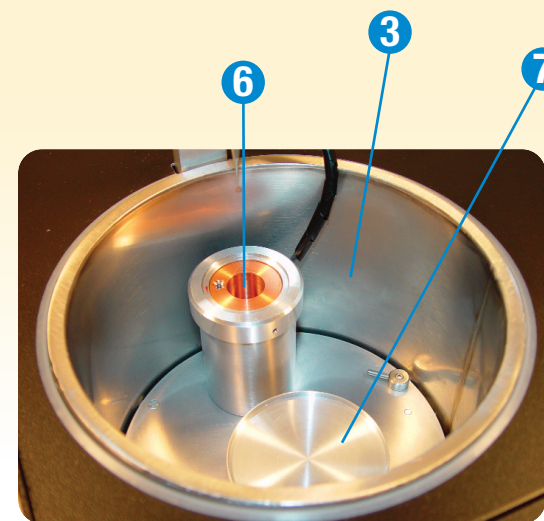
Ultrarapid freezing, when used in conjunction with appropriate accessory processing methods has long been recognized to provide preservation of ultrastructure, localization of soluble inorganic and organic species, and preservation of antigenicity that is superior to that obtainable through conventional chemical fixation techniques and the use of cryoprotectants. Furthermore, it is the only procedure currently available for capturing events that occur on timescales less than a few seconds. Successful ultrarapid freezing requires that heat be withdrawn so rapidly from an unfixed un-cryoprotected sample that no ice crystal damage is visible at the electron microscopic level of analysis. This is not a trivial undertaking, and until recently, the improvements gained through ultrarapid freezing were often paid for in terms of decreasing productivity. However, over the last several years, improvements in methodology and the introduction of refined equipment for the ultrarapid freezing of biological samples has significantly improved both the ease of application of technique and the yield of well preserved samples. The EMS-002 Cryo Workstation allows for a reliable virtually routine freezing of biological samples

Applications:

- Cryo-fixation of virus particles
- Cryo-fixation of isolated or assembled macromolecules
- Cryo-fixation of emulsions and polymers
- Cryo-fixation of suspension and tissues for Cryo-techniques such as freeze fracture/etch, freeze drying, freeze substitution.

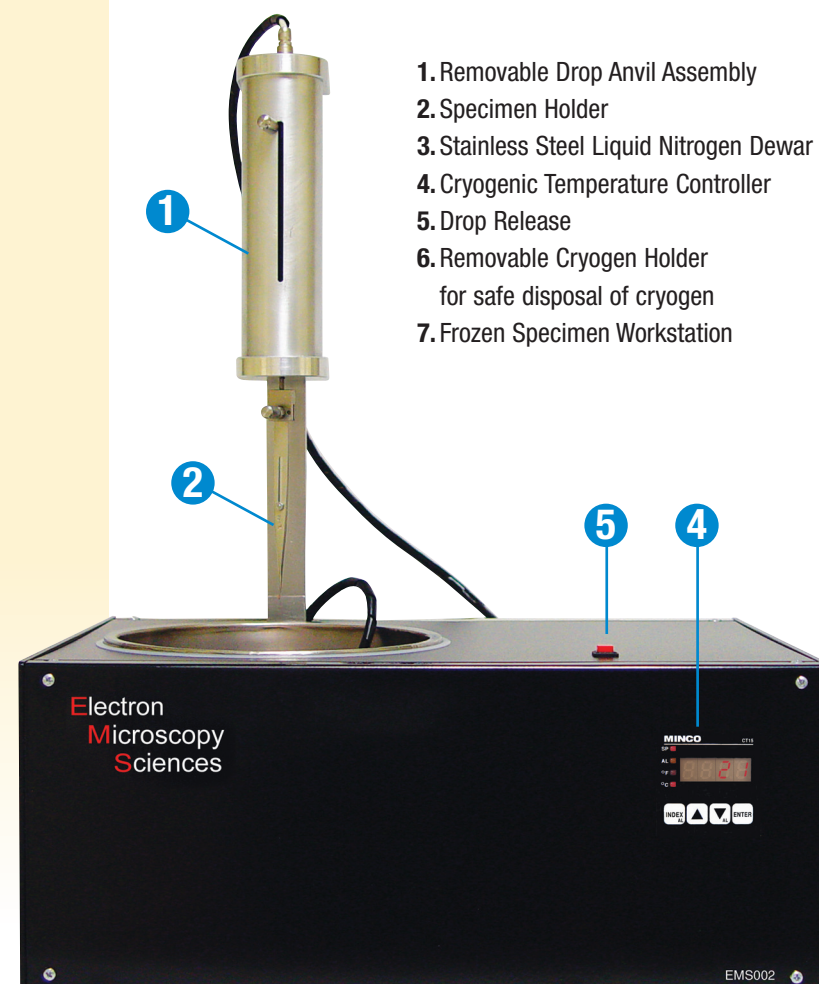
Advantages:

- Economical, universal application rapid freezing unit.
- Plunge freezing with temperature controlled cryogen.
- Frozen samples handled under cold dry nitrogen atmosphere.
- Rapid set up and easy use.
- Reproducible results.
- High and low set points for cryogen control.



Temperature Controller

This allows the user to set desired holding temperatures of the cryogen being used for the freezing process. Easy to use controller also has settable arms to alert the user that the cryogen is warming up and the unit needs attention before the cryogen reach is evaporation temperature.



1. Removable Drop Anvil Assembly
2. Specimen Holder
3. Stainless Steel Liquid Nitrogen Dewar
4. Cryogenic Temperature Controller
5. Drop Release
6. Removable Cryogen Holder for safe disposal of cryogen
7. Frozen Specimen Workstation

Optional Accessories

1. Freeze Substitution Module

Allows the EMS-002 to perform freeze substitution and progressive lowering of temperature (PLT) on a routine basis.

2. Cryo-TEM Transfer Interface

Allows the direct insertion of Gatan cryo-holder into the plunge freezer's liquid nitrogen dewar.

3. Climate Controlled Environmental Chamber

Inhibits air-drying and pre-cooling of samples prior to freezing. Allows easy sample blotting to achieve the optimal amount of sample on your TEM grid.

4. Metal Mirror Impact Freezing Fixture

Allows user to quickly change from the plunge freezing to impact freezing.

5. Diamond Anvil

Special freeze anvil with real diamond plate for rapid freezing and easier specimen removal. Does not require polishing like standard copper anvils.

6. Automatic Liquid Nitrogen Filling

Keeps constant LN₂ level in the Dewar automatically. (Note: To be used only in conjunction with basic plunge freezing or freeze substitution.)

7. Propane Transfer System

Allows the operator to efficiently transfer propane to the condensation chamber from the propane storage vessel.

8. Freeze Forceps

Special tweezers specifically for use with the plunge freezer that are self locking. Extra sets allow quicker turnaround when freezing multiple samples.

9. Muscle Biopsy Clamps

Clamps designed for human muscle biopsies to keep the muscle from contracting during fixation.

Recycling and Operating Costs

The recycling time of the device is less than three minutes, just long enough for it to be ready when you finish processing the last sample. The operating cost is about \$0.47 per sample, depending on the price of liquid nitrogen. The device will operate for about two hours on 10 L of liquid nitrogen, including the initial cool down.

Specifications

Dimensions:	Width — 11 inches (279mm) Depth — 13 inches (330mm) Height — 14 inches (356mm)
Electrical:	100 VAC-240 VAC 50/60 Hz 8A
Temperature Range:	+30 to -200°C
Liquid Nitrogen Consumption:	1 to 2 L per hour
Cryogen Volume:	approximately 20 mL

Process of Ultra-Rapid Sample Cooling

The EMS-002 achieves ultra-rapid cooling by quenching the sample in liquid propane or other suitable cryogen, cooled to near liquid nitrogen temperature. The EMS-002 accomplishes this by using a stainless steel vacuum insulated Dewar and copper condensation chamber. Liquid nitrogen is poured into the Dewar to a level determined by the user. The initial cooling of the cryogen condensation chamber takes about 15 to 20 minutes.

Gaseous propane is then admitted to the condensation chamber slowly enough to allow it to condense and fill the chamber with liquid propane. The propane is led to cool to near liquid nitrogen temperature supervised by the digital temperature controller while the sample is being prepared for freezing. The device requires only about 10 minutes to cool to operating temperature after the first filling of propane, and less than five minutes after subsequent fillings.

The operator can increase or decrease the rate of cooling by adjusting the level of liquid nitrogen in the Dewar. Heaters built into the chamber wall, powered by the temperature controller, evenly warms the propane above its melting point. The controllers' temperature is set for the kind of cryogen being used, for instance, 185°C for commercial grade propane.

Chemically pure propane (map. 190°C) can also be used, but it is significantly more expensive than commercial grade propane and confers no particular advantage. Ethane and a non-flammable cryogen, SUVA-124 produced by Dupont can also be used as a cryogen in this instrument. When using flammable cryogens like Propane & Ethane we recommend using the EMS-002 in a fume hood.

Sample Preparation

The sample can be prepared using any favorite size EM Grid if the system is being used for Cryo-TEM or Panama hat-shaped specimen carriers for Freeze-Fracture or Freeze Substitution. Once the sample is applied to the EM grid for instance, the excess must be carefully blotted away with a small piece of filter paper. Remember, the goal is to form a thin meniscus of specimen across the holes in the grid. Once achieved, insert the specimen holder in the "Drop Anvil" of the EMS-002.

Results and Techniques

The technique provides higher rates of cooling than can be achieved by plunging the sample into the cryogen by hand because of the high velocity and accuracy the sample enters the cryogen. The optimal plunging speed is approximately 100m per/sec it provides more efficient heat exchange. The freezing is fast enough to capture rapid events and labile structures that are not seen in chemically fixed material. The technique has been applied to many types of specimens: aqueous suspensions and emulsions, tissue culture cells, suspensions of a cells, and tissues. Specimens frozen with the EMS-002 may be freeze fractured, freeze substituted, frozen thin sectioned, or freeze dried. The samples may also be used for Cryo-SEM or Cryo-TEM.

Accessories

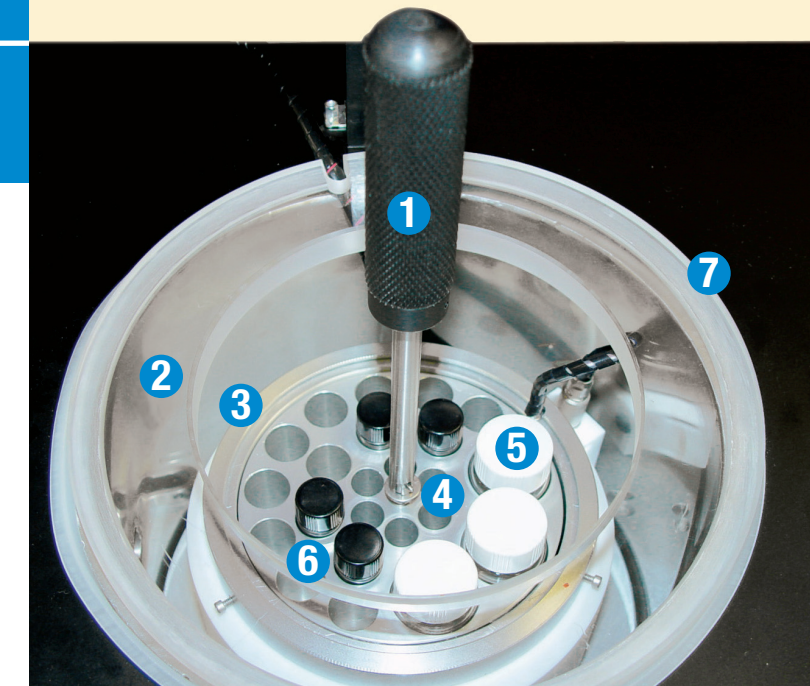
1. Freeze Substitution Module for EMS-002 Cryo Workstation

In the 1980s descriptive ultrastructure analysis essentially came to the end and microscopists found they needed to correlate structure with function to advance their research. About the same time biochemists and molecular biologists were able to see where molecules interacted with cellular membranes. It was essential to marry the work of the structural biologists to the functional biologists by preserving the ultrastructure in the antigenicity of the tissue. During this research it was determined early on that conventional methods of fixation using chemicals were unsuitable for such studies because of the extensive redistribution of proteins, extraction of cytoplasmic constituents, and destruction of cellular antigenicity.

Freeze substitution, which is the dissolution of freezing water (ice) in the frozen specimen at low temperature has turned out to be one of the most powerful techniques in electron microscopy. The dissolution of the ice within the specimen is achieved by substituting water molecules with molecules of an inorganic solvent like acetone, while simultaneously fixing the biological material with Osmium tetroxide and blocks staining with Uranyl acetate. This slow dissolution of the ice takes place over many hours at slowly increasing temperatures. Once the substitution process is complete the samples can be infiltrated and embedded with a wide range of Epoxies or Acrylics. It is even possible to perform a low temperature embedding at -50° Celsius using specialized resins. The end results are beautifully preserved specimens possessing both antigenicity for your immuno-localization studies and perfect structural relationship for descriptive ultrastructure analysis.

The EMS-002 Freeze substitution module is a simple cost-effective method to achieve freeze substitution. Once your samples are frozen on the EMS-002 Cryo Workstation the cryogen condensation module can be removed from the stainless steel Dewar and replaced with the substitution chamber. The freeze substitution controller will control the liquid nitrogen levels automatically as well as the substitution process itself. The user just has to monitor the systems progress during substitution and perform scheduled substitution media changes.

Part # 34765



Freeze Substitution Chamber

1. Transfer tool enables users move the substitution chamber in and out of the substitution chamber while at cryogenic temperatures.
2. Vacuum insulated stainless steel LN₂ Dewar (Part of the Plunge Freezer)
3. Substitution chamber acts as the main heat sink and control interface for the substitution chamber.
4. Substitution holders large mass enhances temperature stability for the samples and media.
5. **Substitution Media Vials Holders:** Allows users to store extra substitution media at the correct process temperature for solution exchanges as required by the protocol being used.
6. **Specimen Vials Holders:** Containers for the tissue being substituted as well as the substitution media.
7. **Substitution Chamber Lid:** Helps maintain Dewar temperature stability and a pure Nitrogen gas atmosphere. Also excludes water vapor from the substitution chamber.

Freeze Substitution Controller

1. **Programmable Substitution Temperature Controller:** Comes preprogrammed from the factory to control precise ramp/soak times and temperatures critical to successful freeze substitution
2. **Liquid Nitrogen Level Controller:** Since the substitution process runs over the course of many days using published protocols, the LN₂ controller insures that LN₂ levels inside the Dewar are maintained at a level appropriate for the process step being executed.

