MiTeGen GraVitri Standard Plunge Cooler

Manual Vitrification System for Cryo-EM Sample Preparation

1. Overview

The MiTeGen GraVitri Standard Plunge Cooler is a manually operated vitrification device designed for preparing cryo-electron microscopy samples. Built on a time-tested design developed in collaboration with the MRC Laboratory of Molecular Biology and Dr. Christopher Russo, this system has enabled researchers worldwide to vitrify thousands of high-quality cryo-EM grids.

The GraVitri Standard model features a passive humidification enclosure with spring-backed blotting rod, providing straightforward and dependable sample preparation with full control over blotting parameters. The system achieves typical plunge speeds of 2 m/s and maintains enclosure humidity above 85%.

This procedure covers setup, operation, and shutdown protocols for safe and effective sample preparation using the GraVitri Standard Plunge Cooler with cryostat.

Note: Images from https://www.mitegen.com/plunge-cooler-series/ showing the GraVitri Standard system, blotting process, and grid transfer should be inserted here for visual reference.

Figure 1. Workflow for sample preparation using the manual plunge cooler. (A) The complete manual plunger assembly. (B) Securing the tweezers (holding the EM grid) to the plunger. (C) Dispensing the sample onto the grid. (D) Blotting the grid to remove excess liquid prior to plunging.

2. Equipment Components

Main Plunge Cooler Unit

- **Plunge Mechanism:** Foot pedal-activated manual plunging system with 72-inch cable release for ergonomic operation
- **Passive Humidification Enclosure:** Environmental chamber that maintains humidity >85% using humidification sponges
- **Spring-Backed Blotting Rod:** Holds blotting paper rounds and provides controlled pressure for sample blotting
- **Tweezers:** Dumont No. 5 tweezers for holding EM grids during sample application and plunging
- **Ergonomic Hand/Arm Rest:** Provides stability during manual sample application and blotting
- **Slotted Base Plate:** Allows precise positioning and movement of the dewar
- **Dimensions:** 11.8" × 11.8" × 31.5" (30 cm × 30 cm × 80 cm); Weight: 25 lbs (11.3 kg)

Cryogenic Workstation

The cryostat dewar contains the following components:

- **Liquid Ethane/Propane Container:** Central vessel holding cryogenic coolant for vitrification (-196°C)
- **Liquid Nitrogen Dewar:** Surrounds coolant container and maintains cryogenic temperature
- **Grid Storage Box:** Cryo-grid box positioned within liquid nitrogen for immediate grid storage
- **Acrylic Base Plate Insert:** Provides stable platform for dewar components

Included Consumables

- Whatman No. 2 blotting paper rounds (50x)
- Humidification sponges (2x)

3. Safety Requirements

Personal Protective Equipment (PPE)

- Laboratory coat
- Nitrile gloves (for general handling)
- Cryogenic gloves (for handling liquid nitrogen and ethane)
- Safety glasses or goggles
- Face mask (as needed)

Hazards

- **Liquid Nitrogen:** Cryogenic liquid (-196°C); risk of cold burns, frostbite, and asphyxiation in poorly ventilated areas
- **Liquid Ethane/Propane:** Highly flammable cryogenic liquid; ensure proper gas cylinder control and work in well-ventilated area
- **Manual Plunging:** Keep hands clear of plunge path; use foot pedal only when ready
- **Cryogen Evaporation:** Always work in well-ventilated area or near fume hood; monitor oxygen levels if available

4. Setup Procedure

1. **Prepare Humidification Enclosure:** Saturate humidification sponges with distilled water and place them inside the passive humidification enclosure. Close the enclosure to allow humidity to build up (target >85% RH).
2. **Install Blotting Paper:** Attach a Whatman No. 2 blotting paper round to the spring-backed blotting rod. Ensure the paper is centered and secure.
3. **Position Plunge Cooler:** Place the GraVitri plunge cooler on a stable work surface. Attach the foot pedal cable release mechanism and ensure the 72-inch cable allows comfortable operation.
4. **Prepare Cryogenic Workstation:** Insert the acrylic base plate into the dewar. Position the liquid ethane/propane container and grid storage box in the dewar according to the system layout.
5. **Fill Dewar with Liquid Nitrogen:** Carefully fill the dewar with liquid nitrogen until it reaches approximately 2-3 cm below the rim. Ensure the grid storage box positions are covered with liquid nitrogen.
6. **Condense Ethane or Propane:** Pre-cool the ethane/propane container by allowing it to equilibrate in the liquid nitrogen for 2-3 minutes. Slowly introduce ethane (or propane) gas into the container. You will hear gurgling as the gas condenses into liquid. Continue until the container is approximately 1/3 to 1/2 full of liquid ethane/propane.
7. **Monitor Cryogen State:** Observe the ethane/propane. It should appear as a clear liquid or slightly slushy mixture. If it begins to freeze solid (white and opaque), stop adding gas and allow it to warm slightly until it returns to a liquid state.
8. **Position Dewar:** Place the dewar on the slotted base plate beneath the plunge mechanism, ensuring proper alignment with the plunge path.

IMPORTANT: Verify that ethane/propane is in liquid state (not frozen solid) before beginning vitrification. Frozen cryogen will damage grids and prevent proper vitrification.

5. Operation Procedure

1. **Check Environmental Conditions:** Verify that the humidification enclosure has reached target humidity (>85%). Allow 5-10 minutes after setup for humidity to stabilize.
2. **Prepare EM Grid:** Mount a freshly glow-discharged EM grid in Dumont No. 5 tweezers. Ensure the grid is held securely but not crushed, and that the carbon film side faces the correct direction for your sample application.
3. **Load Grid into Enclosure:** Carefully open the humidification enclosure hatch and insert the tweezers with the grid. Position the grid horizontally and at the appropriate height for sample application. Close the enclosure partially to maintain humidity.
4. **Apply Sample:** Pipette 3-4 µL of sample onto the grid surface. Work quickly but carefully to minimize evaporation. For best results, apply sample within 30-60 seconds of opening the enclosure.
5. **Manual Blotting:** Rest your arm on the ergonomic hand/arm rest for stability. Using the spring-backed blotting rod, gently touch the blotting paper to the grid surface. Apply controlled, even pressure for 2-5 seconds (adjust timing based on sample viscosity and desired ice thickness). The spring mechanism helps provide consistent pressure.
6. **Plunge:** Immediately after blotting, activate the foot pedal to release the plunge mechanism. The grid will rapidly plunge into the liquid ethane/propane at approximately 2 m/s. Keep your hands clear of the plunge path.
7. **Grid Transfer:** Using pre-cooled forceps or tweezers, carefully remove the frozen grid from the ethane/propane container. Keep the grid submerged in liquid nitrogen at all times during transfer. Place the grid into the cryo-grid storage box while maintaining cryogenic temperature.

8. **Monitor Cryogen Quality:** After several grids, check the ethane/propane for ice crystal contamination (cloudy appearance). If contaminated, carefully remove the container, allow it to warm and evaporate in a fume hood, then prepare fresh cryogen.
9. **Replenish Liquid Nitrogen:** Monitor the liquid nitrogen level throughout your session. Add more as needed to maintain proper coverage of the grid storage area.

Note: *Blotting time is the most critical parameter affecting ice thickness. Start with 3-4 seconds and adjust based on ice quality observed during cryo-EM screening. Longer blotting times produce thinner ice, while shorter times produce thicker ice.*

6. Shutdown Procedure

- **Close Gas Cylinder:** If using an ethane or propane cylinder, ensure all valves are fully closed and secure.
- **Store Frozen Grids:** Transfer the cryo-grid storage box to a liquid nitrogen dewar or cryo-storage system. Ensure grids remain at cryogenic temperature at all times.
- **Evaporate Cryogens Safely:** Remove the ethane/propane container from the dewar and place it in a fume hood to allow safe evaporation. Never dispose of liquid cryogens down drains.
- **Clean Tweezers:** Dry the Dumont No. 5 tweezers with lint-free tissue. Store tweezers in a clean, dry container. Handle with care as precision tweezers are delicate and expensive.
- **Remove Blotting Paper:** Dispose of used Whatman No. 2 blotting paper. The blotting rod can be left installed or removed for storage.
- **Dry Humidification Enclosure:** Open the enclosure and allow it to air dry. Remove humidification sponges and allow them to dry completely before next use to prevent mold growth.
- **Empty and Dry Dewar:** Allow any remaining liquid nitrogen to evaporate in a well-ventilated area or fume hood. Once empty, wipe down the interior with a lint-free cloth and allow to air dry completely.
- **Store Equipment:** Store all components in a clean, dry location. Ensure the plunge mechanism is in the up position to prevent stress on the release mechanism.

7. Troubleshooting & Tips

Optimizing Blotting Parameters

Starting parameters for standard grids: 3-4 seconds blotting time with gentle, consistent pressure. Blotting time has the greatest influence on ice thickness. For thicker samples or viscous solutions, increase blotting time to 4-5 seconds. For thin, low-concentration samples, reduce to 2-3 seconds. The spring-backed blotting rod provides natural pressure control, but manual technique still matters for consistency.

Adjust parameters based on ice quality observed during cryo-EM screening sessions. Optimal ice shows clear particle distribution without excessive thickness or holes.

Humidity Management

The passive humidification system relies on saturated sponges to maintain >85% humidity. If humidity drops during long sessions:

- Re-saturate humidification sponges with distilled water
- Minimize opening of the enclosure between grids
- Work quickly during sample application to reduce exposure time

Cryogen Temperature Control

Proper ethane/propane consistency is critical for high-quality vitrification:

- **Optimal state:** Clear liquid or slightly slushy consistency
- **Too frozen (white, opaque):** Allow to warm slightly by removing from deepest part of liquid nitrogen
- **Contaminated (cloudy):** Replace with fresh cryogen
- **Temperature monitoring:** Ethane should be maintained between -183°C and -170°C for optimal vitrification

Grid Handling Best Practices

- Always use freshly glow-discharged grids (within 30-60 minutes of discharge)
- Pre-cool all tools that will contact frozen grids in liquid nitrogen
- Never allow frozen grids to warm above -150°C during transfer
- Use the ergonomic hand/arm rest to maintain steady, controlled movements during sample application and blotting
- Dumont No. 5 tweezers are for grid handling ONLY - never use for other tasks to prevent tip damage

Common Issues & Solutions

Problem: Grids too thick or samples not spreading properly

- Solution: Increase blotting time by 0.5-1 second increments or apply slightly more pressure during blotting

Problem: Grids too thin or showing holes

- Solution: Decrease blotting time by 0.5-1 second increments or reduce blotting pressure

Problem: Inconsistent ice thickness across grids

- Solution: Ensure blotting paper is flat and centered; practice maintaining consistent pressure and timing; check that humidity is stable

Problem: Ice crystal contamination on grids

- Solution: Replace ethane/propane; ensure rapid transfer from plunge to storage; maintain proper liquid nitrogen levels

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References:

- MiTeGen GraVitri Plunge Cooler Series: https://www.mitegen.com/plunge-cooler-series/