MiTeGen
www.mitegen.com

GraVitri Plunge Cooler Series

User's Manual v1.1

Table of Contents

Table of Figures

Overview Schematics

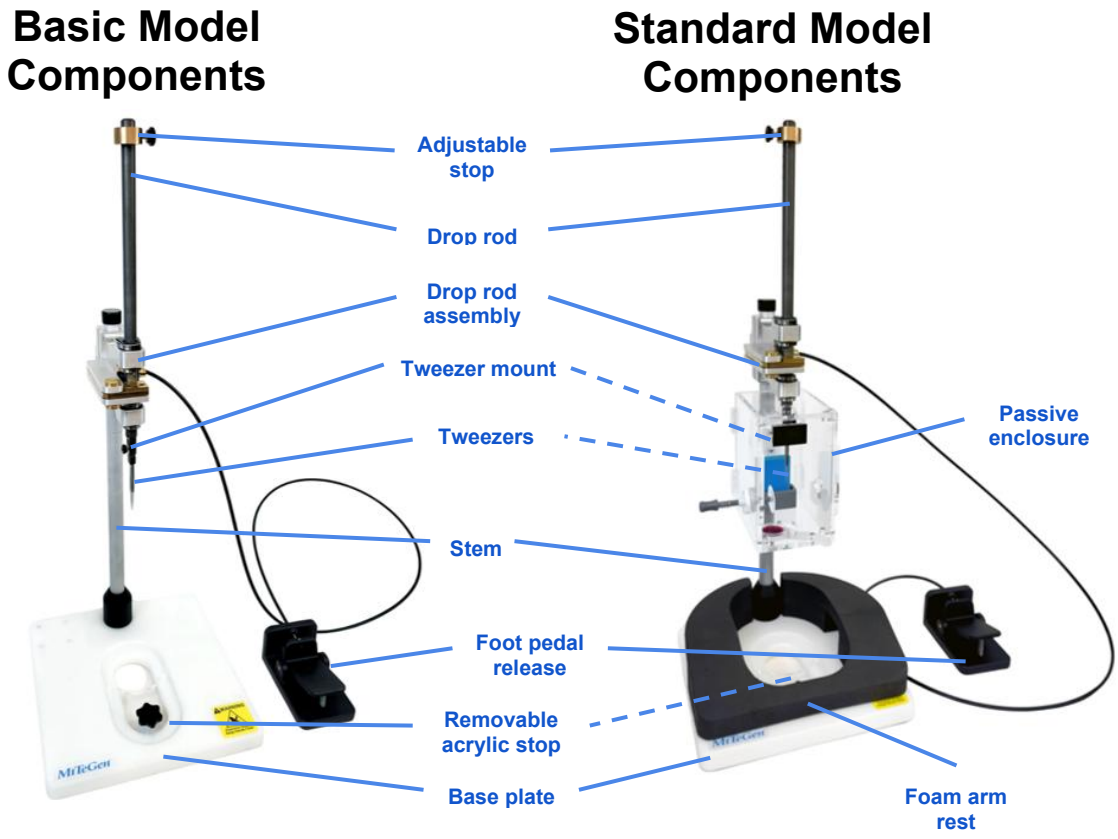

Figure 1: Overview schematics of Basic (left) and Standard (right) models

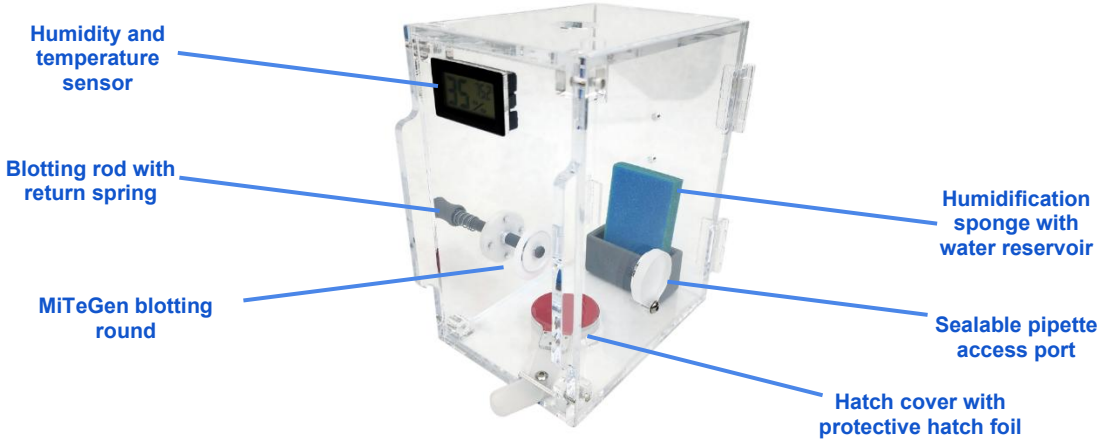

Figure 2: Overview schematics of passive enclosure

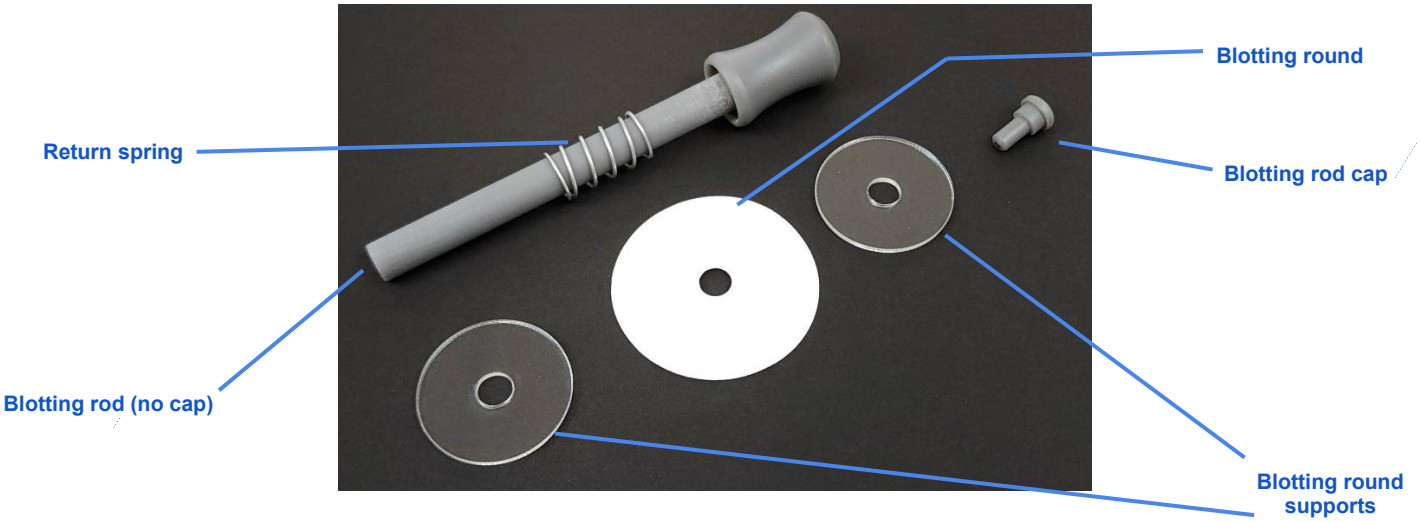

Figure 3: Overview schematics of blotting rod assembly

Handling and Operational Precautions

Caution: All users must read this manual, become aware of the hazards, and be properly trained before use.

Notice: This manual covers the specific usage of the plunging equipment. The equipment is designed to only be used in facilities and labs where there are policies and procedures regarding the safe use and disposal of liquid nitrogen and flammable cryogens, and where the facility has provided to the personnel the training necessary for such safe usage and disposal. The laboratory or facility is responsible for providing a proper working environment and acceptable safety equipment.

Warning - Extreme cold: Liquid nitrogen (LN2) can rapidly freeze skin tissue and eye fluid, resulting in cold burns, frostbite, and permanent eye damage even by brief exposure. These risks are even more acute for liquid hydrocarbons, for which the protective Leidenfrost effect is greatly reduced. Wear thick clothes and protective gear when working with liquid cryogen. Do not wear shorts, short-sleeved shirts, or open footwear when working with liquid cryogen to avoid harm. Safe attire includes: long pants, eye protection, cryogenic gloves, and additional protective clothing when necessary.

Warning - Asphyxiation: It is very important to provide adequate ventilation in areas using liquid nitrogen. Liquid nitrogen expands 695 times in volume when it vaporizes and has no warning properties such as odor or color. Hence, if sufficient liquid nitrogen is vaporized, so as to reduce the oxygen percentage to below 19.5%, there is a risk of oxygen deficiency which may cause unconsciousness. Death may result if oxygen deficiency is extreme. To prevent asphyxiation hazards, handlers have to make sure that the room is well ventilated when using cryogens indoors. A minimum of six air changes per hour is suggested in these areas. Provide oxygen monitoring for areas where oxygen displacement may occur.

Warning: The tweezers are extremely sharp, handle with care. When not in use, the protective cover should be placed over the tips.

Warning: The cryogens used in the cryogen cup (ethane, ethane mixtures most often) are typically extremely flammable. Conform to all lab and facility rules and safety procedures for using flammable cryogens and gases. Dispose of LN2 and cryogen according to all lab and facility safety procedures.

Warning: The sharp tweezers are dropped at high speeds. Keep hands clear of the plunge path when in operation.

Caution (*Standard only*): Plunging the rod while the bottom hatch of the acrylic enclosure is closed can damage the tweezers and/or the enclosure. Always move the hatch cover out of the plunge path before plunging, and leave it open at all times when the enclosure does not need to maintain high humidity.

Caution: As much as possible, only move the drop rod while depressing the foot pedal.

Caution: Any type of alcohol can cause acrylic plastic to crack. Do not use any type of alcohol to clean the acrylic humidity chamber. The recommended cleaner is dish soap and a soft cloth or sponge, the recommended disinfecting solutions are diluted household bleach or 3% hydrogen peroxide.

Installation

STEP 1. Remove all parts from packaging and place them on the flat surface that you will be working on. **Note (*Standard model only*): DO NOT DISPOSE** of the rectangular blue piece of foam inside of the acrylic enclosure. It is used to humidify the chamber during operation, and is not packaging.

STEP 2. Clip the zip ties holding the control cable. **CAUTION:** The cable has a spring force. Hold the cable as you clip the zip ties then gently unwind it to avoid injury. **Note:** If the cable housing is loose, adjust the brass cable screw on the side of the drop rod assembly until the housing does not slide freely along the cable.

STEP 3. Use the included 5mm hex key and M6 socket screws to attach the stem rod assembly onto the base plate (see Figure 4 below). The drop rod assembly on top of the stem should rest over the base plate when screwed in.

Figure 4: Assembly of stem rod assembly and base plate

STEP 4. ***Standard model only.*** With the included 2.5 mm hex key, mount the passive enclosure to the stem using the three M4 screws and washers. (See Figure 5 below). Place the washer onto the screw and insert through the inside of the enclosure to mount the enclosure to the stem. **Note:** newer models may have a black enclosure mount instead of a white one. The instructions for installation remain the same.

Figure 5: Assembly of passive enclosure and stem

STEP 5. Unscrew the small thumb screw from the tweezer mount. Press and hold down the foot pedal (or squeeze the handbrake-style release) to draw back the rod release mechanism, then gently slide the rod down through the bearings until the release plate aligns with one of the grooves in the rod (see Figure 6 below). Release the foot pedal (or handbrake-style release) and the rod will be retained in place. Re-insert the previously removed thumb screw once the rod is in place.

Figure 6: Release plate aligned with groove in drop rod

Operation

Warning: Safety glasses required from this point

STEP 1. Insert ethane Dewar/cryostat into base plate as shown in Figure 1 above, and then insert the acrylic stop to fixture in place

STEP 2. Prepare the liquid ethane. If using the MiTeGen Cryostat and Temperature Controller, please refer to the user manual for these products. **Note:** While these operation instructions refer explicitly to liquid ethane, they are equally applicable to all commonly used hydrocarbon-based cryogens (e.g liquid propane, liquid ethane-propane mixture, etc.)

STEP 3. Load grid boxes into grid box holders, as many as are needed to store the number of grids to be plunged. **Note:** When loading grids into grid boxes, only one hand will be free to perform manual tasks. Thus, prior to plunge, prepare a grid box to receive the next plunged grid without additional manual intervention.

STEP 3.5 (OPTIONAL). Insert dark blue foam ring into Dewar/cryostat as is shown in Figure 7 below. **Note.** We have had users report successful sample preparation both with and without foam ring use. We advise users to try

both to discover what works best for them and their protocol. The following instructions apply equally to both, though provided figures omit the foam ring for the sake of image clarity.

Figure 7: Insertion of dark blue foam ring into ethane Dewar/cryostat

STEP 4. Following grid pre-treatment (e.g. exposure to air plasma, addition of affinity layer onto grid foil), use the provided tweezers to hold a grid. **Note *(Standard model only)***: Open both enclosure doors.

STEP 5. Slide the back end of the tweezers into the tweezer mount, and use the small thumb screw to secure them in place (see Figure 8 below).

Figure 8: Fixturing of tweezers in tweezer mount

STEP 6. ***Standard model only:*** With enclosure doors open, slide the return spring onto the blotting rod. Then, without cap, slide the blotting rod through the

appropriate port on the enclosure side door (please refer to Figure 2) with the rod's female end sticking into the enclosure. Open the door and slide the blotting round supports, blotting round, and cap onto the end of the blotting rod (see Figure 9 below). Close the door.

Figure 9: Correct assembly of blotting round and blotting rod through the side door

STEP 7. ***Standard model only:*** Wet the blue humidification sponge with **WARM** water, and fill ¾ of the water reservoir with **WARM** water. Place the reservoir with the sponge in it to the back of the enclosure and close both doors. Slide the hatch cover to the closed position if it is not there already. The enclosure should now look like Figure 2 above. A humidity reading of >85% RH should occur within a few minutes of all doors and hatches being closed. **Note:** None of the enclosure's doors or hatches should be opened after this point unless expressly mentioned in the proceeding instructions. Failure to ensure this can result in large, sudden drops in enclosure humidity, which can lead to excess sample drying and changes to sample concentration. As condensation on the humidity sensor in the saturated enclosure can cause a delay in the detection of sudden humidity decreases, a high humidity reading, in this situation, does not guarantee

adequate enclosure humidification. When in doubt, repeat this step, ensuring not to open any doors or hatches unless expressly instructed.

STEP 8. Load sample into pipette. ***Basic model*****:** Apply sample directly to the foil-side of the grid. ***Standard model*****:** Open the door to the sealable pipette access port and insert the loaded end of the pipette. Apply your sample to the foil-side of the grid (see Figure 10 below). When finished, close the door to the pipette access port.

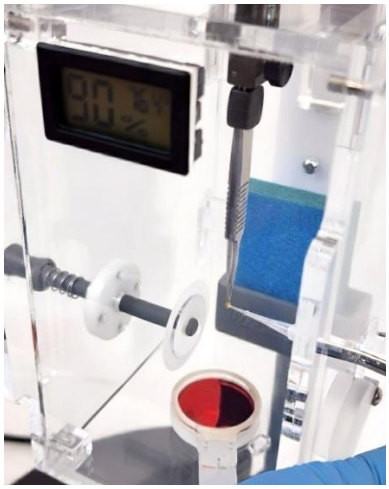

Figure 10: Loading sample onto grid

STEP 9. ***Basic Model:*** Using filter paper, gently blot excess solution from the grid. ***Standard model:*** Use the blotting rod assembly to remove excess liquid from the grid as shown in Figure 11. While the return spring should automatically retract the blotting rod, be sure the **blotting rod is fully retracted prior to plunging your sample.** Failure to do so can result in damage to the blotting rod, the grid, the tweezers, and/or other instrument components. **Note:** Both units are designed for free rotation of the drop rod without change in drop rod height in order to accommodate both front side and back side blotting. For the former, rotate the drop rod 180° between sample application and blotting. For the latter, do nothing after sample application.

Figure 11: Removal of excess sample using blotting rod assembly

STEP 10. ***Standard model only:*** Slide open the enclosure's hatch cover as shown in Figure 12 below.

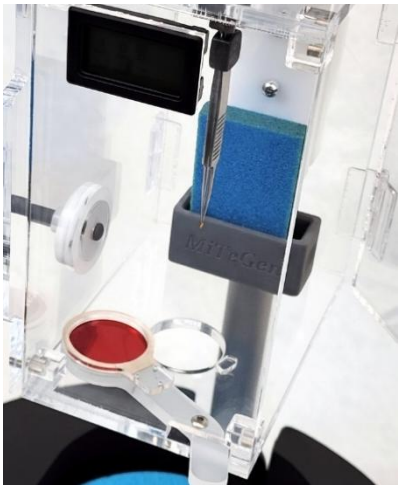

Figure 12: Opening the hatch cover to prepare for grid plunging

STEP 11. Press down firmly on the foot pedal (or squeeze the handbrake-style release tightly) to plunge your sample. Release the foot pedal (or handbrake-style release) only after the plunge is complete.

STEP 12. Ensure that the liquid nitrogen level in the Dewar/cryostat is equal to or above the terminal height of the grid after drop. Ensure, along the major axis of the machined slot in the base plate, that there are no grid boxes or grid box holders, in order to preclude grid damage during the hop from liquid ethane to liquid nitrogen.

STEP 13. Remove the acrylic stop. Grasp the Dewar/cryostat with one hand to prepare to slide it along the machined slot in the base plate. Place the other hand on the drop rod's adjustable stop. When ready, quickly raise the drop rod such that the grid exits liquid ethane, and just clears the top ethane cup surface. Slide the Dewar/cryostat away from the drop rod, and push down on the adjustable stop such that the tweezer-held grid plunges directly into liquid nitrogen. This process should be completed in <1s in order to ensure no sample devitrification.

STEP 14. ***Basic model:*** Hold onto the tweezers, then loosen the thumb screw that is holding them in place, as shown in Figure 13. Once the tweezers are released from the tweezer mount, use one hand to carefully raise the drop rod up while the other hand holds the tweezers in place or lowers them slightly out of the tweezer mount. Make sure the grid remains below or very close to the liquid nitrogen surface for the duration of this process. **Note:** The release plate mechanism is calibrated to ensure that the drop rod can be held statically, for short periods of time, anywhere along its length regardless of groove location on the drop rod. If, after firm pressure is released from the foot pedal, it is observed that the drop rod is not being held in place, please use the contact info at the end of this manual to contact MiTeGen support. ***Standard model****:* Position the foam armrest as shown in Figure 1 above. Rest forearms on either side of the armrest such that hands can comfortably hold the tweezers and manipulate and move the tweezer mount. Once set, remove the tweezers from the tweezer mount as is described above for the ***Basic model****.*

Figure 13: Removing tweezer-held grid from drop rod after liquid ethane plunge

STEP 15. Carefully move the grid into a free grid box slot without the grid rising more than a few millimeters above the liquid nitrogen-air interface (see Figure 14). Once inside a free grid box slot, open the tweezers to allow the grid to rest inside the slot.

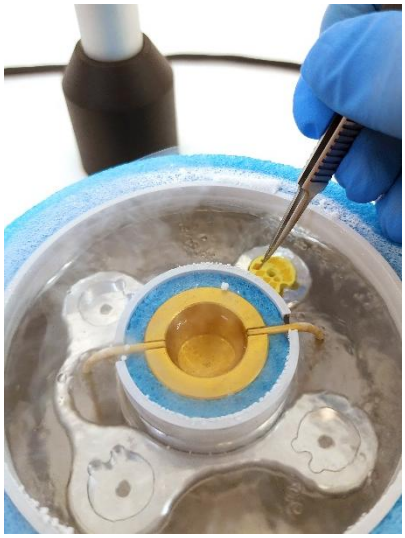

Figure 14: Movement of plunged grid into empty grid box slot

STEP 16. Warm the tweezer tips. Press the foot pedal (or squeeze the handbrake-style release) and slide the drop rod up to its pre-plunge position. Repeat steps 4-15 until all grids have been plunged.

We look forward to hearing about the research you perform using this equipment.

For technical assistance, please contact us:

support@mitegen.com

1-607-266-8877

www.mitegen.com