

**※ Violation of the rules would result in a warning from BioCRF and restricted use of the facilities for the whole research group ※**

**How to Become a User**

1. The Cell Culture Facility is open to HKUST users only.
2. All the equipment in the Cell Culture Facility is for mammalian cell culture use only and is not catered for any other applications.
3. Attend the courses “Safety Orientation for Laboratory Personnel” and “MC06 Biological Safety” organized by HSEO and obtain the course completion certificate for “MC06 Biological Safety”.
4. Download the application form “Application for Access to Cell Culture Facility” from [www.biocrf.ust.hk/cell-culture](http://www.biocrf.ust.hk/cell-culture), under the session “How to apply for access”.
5. Send a scanned copy of the completed application form and the “MC06 Biological Safety” course completion certificate to BioCRF staff via email (Cherry Leung, [cherry1cl@ust.hk](mailto:cherry1cl@ust.hk)).
6. Book a cell culture facility training session via BioCRF’s [booking system](#). The operation of equipment and the basic technique for adherent cell subculturing will be demonstrated. Attendance of the whole training session is compulsory and irrespective of the applicants’ experience in cell culture.
7. Arrange an evaluation session with BioCRF staff via email (Cherry Leung, [cherry1cl@ust.hk](mailto:cherry1cl@ust.hk)) on the operation of equipment and the basic technique for adherent cell subculturing. The passing criteria will be provided during the training.
8. HeLa cells in a 10 cm dish will be provided for subculture evaluation. Users should bring along all other necessities (e.g., tools, reagents, and consumables) for the subculture evaluation, including but not limited to DMEM, PBS, Trypsin-EDTA, and cell culture dishes.
9. Users are allowed to book the Biosafety Cabinets (BSCs) via BioCRF’s [booking system](#) after passing the evaluation.
10. Users are required to re-submit the application form “Application for Access to Cell Culture Facility” biannually, but there is no need to attend the training and evaluation sessions upon re-submission.
11. BioCRF’s cell culture facility is operated on a first-come-first-served basis. No priorities will be given to a particular department/ research group.

## General Rules

1. All safety- and hygiene-related issues will be taken seriously. Users who fail to maintain good personal and environmental safety/hygiene will be required to stop their experiments immediately. The access to the facility by the entire research group will be restricted until all issues are resolved.
2. BioCRF reserves the right to immediately terminate any research activities in the cell culture room. Users can consult BioCRF staff in advance to determine whether their experiments are allowed and clearly describe their research activities in the form “Application for Access to Cell Culture Facility” for approval.
3. Users must attend a cell culture training session and an evaluation session organized by BioCRF staff; and the “Safety Orientation for Laboratory Personnel” and the “MC06 Biological Safety” course organized by HSEO before using the cell culture facility.
4. NO one is allowed to use the cell culture facility before passing the evaluation by BioCRF staff.
5. Booking must be made through BioCRF’s [booking system](#) before using the cell culture facilities.
6. The form “Application for Access to Cell Culture Facility” should be completed biannually. Send a scanned copy of the completed application form to BioCRF staff for approval via email (Cherry Leung, [cherrylel@ust.hk](mailto:cherrylel@ust.hk)).
7. Always wear proper laboratory clothing and personal protective equipment, e.g. a clean lab coat, gloves, etc.
8. No storage facility is provided in the room. Users should take away all their belongings after their experiments.
9. Users have the responsibility to maintain the overall cleanliness of the room.
10. Report all accidents to BioCRF staff irrespective of their severity.
  - In the event of an exposure incident, first aid procedures must be initiated immediately. Wash the exposed area with soap and water, or flush mucous membranes with water for 15 minutes. The eyewash is in Room 6141A next to the sink.
  - Contact BioCRF staff after the first aid procedures are initiated.
  - All work-related exposures and incidents must be reported to HSEO via the Incident/Accident Report Form (available on HSEO’s website).

## Equipment Usage

### CO<sub>2</sub> Incubators

1. Each user will be assigned a shelf in a specific incubator. Cultures can only be stored on the assigned shelf.
2. All culture flasks, dishes, and plates should be sprayed with 70% ethanol before they are transferred to the assigned shelf in the incubator.
3. All culture flasks, dishes, and plates should be labeled properly including name, cell type, and date using a permanent or oil base marker. Any unidentified cultures will be disposed of without prior notice.
4. Cultures will be discarded after 7 days. If a longer-than-7-day culture time is required, please inform BioCRF staff for case-by-case approval.
5. Any cultures showing a sign/ risk of biological contamination (e.g., cloudy medium) will be discarded immediately without prior notice.
6. Incubators are kept at 37°C and 5% CO<sub>2</sub>. Users are NOT allowed to adjust the settings.
7. Report all contamination issues to BioCRF staff immediately.
8. In case of biological spills in the incubators,
  - If the spill is restricted to only the shelves, spray 1% SDS 70% ethanol onto paper towels and wipe the soiled surface. Then, wipe the surface with 70% ethanol at least 3 times until it is not slippery.
  - If the contaminated area is large, or the spill has reached the humidity pan, inform BioCRF staff immediately for decontamination.
  - Do NOT disinfect with bleach.
9. Make sure the inner door is closed and locked before closing the external door of the incubators.
10. Disinfection of the incubator will be performed each month. Notice will be placed on the incubators to be disinfected. Please transfer the cultures to the backup incubator before the disinfection day.

### Biosafety Cabinets (BSCs)

1. BSCs should only be used after sterilization with UV light, wiping with 1% SDS in 70% ethanol, and wiping with 70% ethanol at least 3 times until the surface is not slippery.
2. Always spray your gloves with 70% ethanol before and after your work in BSCs.
3. All items should be disinfected with 70% ethanol before transferring into the BSCs.
4. NO flames are allowed inside the BSCs.
5. Bleach should NOT be used to clean the BSCs.
6. Turn off the vacuum pump after use.
7. No solution residue should remain inside the vacuum tubing.
8. If the waste level has reached the 3L mark, empty the waste bottle of the vacuum pump. Replenish with 300mL tap water and 300mL bleach.
9. Fill in the logbook before leaving.

### Inverted Optical Microscope

1. Select the objectives by pressing the Obj. switch button on the operation panel. Do NOT rotate the nosepiece (turret) manually which will damage the motor.
2. Turn off the light source after use.
3. For disinfection, spray a paper towel with 70% ethanol and wipe the microscope stage and focus knob. Do NOT spray 70% ethanol directly onto the microscope.

### Centrifuge

1. The centrifuge should be used for cell culture purposes only.
2. The maximum centrifuge time per run is 10 minutes.
3. Make sure the tubes are balanced across the center of rotation.
4. Only use centrifuge tubes (15mL/ 50mL) and microplates.
5. Do NOT remove the centrifuge buckets from the centrifuge.
6. In case of biological spills in the centrifuge or the swing buckets, spray 1% SDS 70% ethanol onto paper towels and wipe the soiled surface. Then, wipe the surface with 70% ethanol at least 3 times until it is not slippery. Do NOT disinfect with bleach. Users can disassemble the parts for easy cleaning but they should assemble all the parts properly to avoid damaging the centrifuge.

### Water Bath

1. The water bath is kept at 37°C. Users are NOT allowed to adjust the temperature.
2. Refill with Milli Q water if necessary. Do NOT use tap water.
3. Turn off the water bath when it is idle.

### **Waste Disposal**

1. A biohazard bin is provided in the cell culture room for the disposal of contaminated solid waste. Liquid waste and sharps should NOT be discarded in this bin.
2. Paper towels and gloves without biohazards should be discarded in regular bins in the cell culture room.
3. 10% bleach must be added to contaminated liquid waste before disposal to the sink. Discard the decontaminated waste under running water and rinse the sink with water afterwards.
4. A broken glass collection box is provided in the room. Only broken glass can be discarded in this box. Contact BioCRF staff for immediate clean-up. If the glass is contaminated (e.g., with cells), 10% bleach should be added to the box for disinfection.

### **Dealing with spills OUTSIDE incubators, BSCs and centrifuge**

1. Apply paper towels then disinfectant (10% freshly prepared bleach) to the spill, allowing a 20-minute contact time. Repeat once (to reach a total contact time of 40 minutes).
2. Wipe dry with paper towels. Discard used paper towels into regular bins. Bleach-tinted materials have to be placed into a bag then transferred to a regular bin.
3. Rinse the affected surfaces with sterile water at least 5 times to remove the bleach completely.
4. Inform BioCRF staff about the spill.

### **Checklist before leaving the room**

1. If used, clean the vacuum tubing by aspirating around 20mL of 1% SDS in 70% ethanol. Ensure there is no residual solution in the vacuum pipe. Shut down the pump.
2. Remove all your belongings from the BSC.
3. Disinfect and wipe the BSC with 1% SDS in 70% ethanol and then 70% ethanol at least 3 times until the surface is not slippery.
4. Close the sash and turn on the UV light for disinfection. Check the UV timer which should count down from 30 minutes.
5. If the waste level has reached the 3-Litre mark, empty the waste bottle of the vacuum pump. Replenish with 300mL tap water and 300mL bleach.
6. Turn off the microscope, water bath, and centrifuge if they are idle.
7. Fill in the logbook.