



iPSC Facility

Overview

The iPSC Facility is a controlled sterile environment dedicated exclusively to the reprogramming, maintenance, expansion and differentiation of induced pluripotent stem cells (iPSCs) and their derivatives.

The facility is equipped with biological safety cabinets, incubators with controlled oxygen and carbon dioxide atmosphere, fluorescence microscopy system, centrifuge, water bath and dedicated refrigerator for reagents and media.

Due to the high sensitivity of pluripotent stem cells to contamination and environmental changes, strict aseptic procedures and facility-specific regulations must be followed at all times.

Equipment

Biological Safety Cabinets:

- SafeMate EZ 1.2LED , Class II MSC (BioAir)
- SafeMate EZ 1.2LED , Class II MSC (BioAir)

Incubators:

- Steri-Cycle i160, CO₂ Incubator (Thermo Scientific)
- Steri-Cycle i160, CO₂/N₂ Incubator (Thermo Scientific)

Microscope:

- ZEISS Axiovert 5 Light Widefield Microscope (with AxioCam)
 - Brightfield and fluorescence imaging
 - Available filtersets: DAPI/FITC/TRITC/Cy5
 - Available magnifications: 5x, 10x, 20x, 40x (all dry objectives, no immersion)
 - Available camera: Zeiss AxioCam 202 mono (resolution: 1920 x 1080, pixel size: 5.86 um x 5.86 um)

Other Equipment:

- Benchtop refrigerated centrifuge SL8R (Thermo Scientific)

- Water bath WB22 ARGOLab
- Refrigerators/freezers (+4°C / -20°C, only for working solutions, not intended for long-term storage)

Location

The iPSC Facility is located on the 1st floor, east side, GEOTEC 02 (013-015).

Opening hours

Monday to Sunday: H24

Technical assistance is not guaranteed outside working hours.

Please note that, according to the Safety Guidelines of the University of Padua:

“Do NOT work alone outside of working hours, particularly in cold rooms, or in rooms with X-ray sources and in cases of complex and dangerous operations. Always check whether specific procedures require special attention (especially for the cold room).

The presence of undergraduate students is contingent on the presence of at least one member of structured staff”

Service Coordinator

Prof. Agnese De Mario

Staff

Dr. Michela Menegollo - Dr. Valeria Bertin - Dr. Marco Pistore

The Facility's staff are available Monday to Friday during normal working hours (09:00-16:00).

For any information or requests, please contact the facility staff at the following e-mail address:

CN3.biomed@unipd.it

Guidelines

Access and training

THE ACCESS TO THE ROOM IS ALLOWED ONLY UPON COMPLETION OF THE REQUIRED TRAINING.

Please contact the staff at **cn3.biomed@unipd.it** prior to the first access.

Access is restricted to personnel directly involved in experimental activities.

Booking procedures

The hoods must be reserved before every work session.

The reservation includes the use of an incubator, centrifuge, microscope, waterbath.

Reservations are made through the dedicated page on the DSB Facilities & Services website by selecting the “book now” icon associated with the required instrument. When making your reservation, indicate the **name of the user**, the **group leader**, and **lab phone number**.

If your plans change, reservations may be cancelled up to 24 hours before the start of the reserved time slot.

On the booking webpage, hoods are identified by specific numbers that you can find also on the instruments:

- **hood n.1:** SafeMate EZ 1.2LED , Class II MSC (BioAir)
- **hood n.2:** SafeMate EZ 1.2LED , Class II MSC (BioAir)

Please note:

- Minimum booking slot: 30 min per user per day
- Maximum booking time: 4 hours per user per day.
- Longer reservations require prior approval from the Facility Staff.
- Late cancellations or no-shows may be charged as completed reservations.
- If necessary, reservations can be cancelled no later than 24h prior to use.

Rules

1. GENERAL

- Upon completion of mandatory training, users will be added to the facility mailing list for communications regarding maintenance, contamination events and facility updates.
- Consumables are NOT provided by the facility; users are expected to bring their own required materials.
- Eating, drinking and food storage are strictly forbidden.
- Any equipment malfunction or damage must be immediately reported to the Facility Staff. The last user booked will be held responsible for the equipment status.
- To maintain a contamination-free environment and ensuring the quality of pluripotent stem cell cultures, USERS MUST FOLLOW GOOD CELL CULTURE PRACTICE AT ALL TIMES:
 - Inside the room, it is mandatory to use a dedicated lab coat and disposable gloves.
Please note: **lab coats used inside the facility must not be worn outside the room!**
 - Wash hands before entering the facility.
 - Disinfect all materials before placing them inside the hood.
 - Work rapidly and efficiently.
 - Minimize exposure of sterile materials to room air.
 - Avoid unnecessary talking while working and in front of the incubators.
 - Never touch sterile surfaces with non-sterile objects.
 - Use sterile disposable pipettes and consumables.
 - Maintain an uncluttered workspace.
 - Avoid disrupting laminar airflow.
- All equipment has to be kept CLEAN and SWITCHED OFF after use. **Before leaving the facility, all users MUST DISINFECT ANY SURFACE OR EQUIPMENT TOUCHED DURING THEIR WORK using 70% ethanol.**
- DO NOT leave anything in the room and inside the hoods and water bath.
- Check if you are the last user of the day and, in this case, ensure that:
 - Water bath and microscope are switched off.
 - Hoods are properly closed and switched off.
 - Incubator doors are fully closed.

- Lights are switched off.
- Report to the staff the presence of incorrect behavior or waste not properly disposed of.

2. **CELL LINES AND CULTURE**

➤ **MYCOPLASMA TESTING:**

- **All new PSC/iPSC lines and fibroblast of human or murine origin introduced into the facility must be tested NEGATIVE FOR MYCOPLASMA before use.**
- All cell lines maintained in the facility must be registered in the facility database
- **Routine mycoplasma testing** is mandatory and will be performed **every 15 days**, according to the facility's procedures.
Failure to comply with testing requirements may result in suspension of facility access.
- Users are responsible for the daily monitoring of their cultures.
- Any contamination event must be reported immediately to the Facility Staff.

➤ **MOVEMENT OF CELL CULTURES:**

To minimize contamination risks:

- Cell culture plates originating from other cell culture rooms are NOT allowed inside the facility.
- Plates, flasks or cultures removed from the facility can not re-enter the facility.
- Exceptions (e.g. cell sorting, viral transduction or other specialized procedures) require prior approval from the Facility Staff.

3. **SPACES AND EQUIPMENTS**

➤ **BIOLOGICAL SAFETY CABINETS (HOODS):**

Before your session:

- Verify that the work area is clean.
- Disinfect all materials entering the hood with 70% ethanol.

Only materials necessary for the current procedure should be placed inside the hood, to avoid laminar airflow block.

At the end of your session:

- Remove all materials.
- Clean all surfaces thoroughly using 70% ethanol.
- Switch on UV sterilization for 15 minutes.

Any spill must be cleaned immediately and reported when appropriate. If the leakage spreads under the inner steel surface of the hood, it must be removed and the surface below cleaned.

➤ INCUBATORS:

Cultures stored in incubators must be clearly labelled with:

- **User name**
- **Cell line identification**
- **Date**

Users are responsible for maintaining incubator cleanliness.

Contamination events involving incubators must be reported immediately. Deep cleaning and sterilization procedures may be required following contamination events.

Avoid unnecessary opening of incubator doors and avoid talking while the incubator is open.

If not used, incubators should be turned off; a sterilization cycle has to be done once restarted, prior to use.

Current user contacts have to be specified on the incubator door.

➤ MICROSCOPE:

The ZEISS Axiovert 5 microscope may be used for routine monitoring of iPSC cultures and fluorescence imaging.

Users must:

- Receive training before first use.
- Export all acquired images immediately after acquisition.
- Clean the stage (objectives and plate) after use.

Turn off illumination systems and accessories after use.

Long fluorescence acquisition sessions must be reserved in advance.

➤ WATER BATH

The water bath available in the room is filled with distilled water. Before using it, please CHECK the water level. Remember to SWITCH IT OFF AFTER USE.

➤ CENTRIFUGE

Any spill must be cleaned immediately and reported to the Facility Staff.

➤ REFRIGERATORS:

Storage areas are assigned by the facility staff.

All stored materials must be labelled with:

- User name.
- Laboratory name.
- Date.

Expired or abandoned materials may be removed by facility staff.

4. WASTE MANAGEMENT

Each user is responsible for the proper disposal of biological waste.

➤ LIQUID WASTE:

Aspiration system is not available. Liquid biological waste must be collected into falcon tubes or small bottles and disposed of in designated bins.

➤ SOLID WASTE:

Solid biological waste must be disposed of in designated containers.

Waste containers must never be overfilled. Once full, or in the event of an extended period of inactivity in the room, containers must be sealed and handed over to the Safety service for proper disposal, according to the facility's procedure.

5. CONTAMINATION EVENTS

In the event of bacterial, fungal or mycoplasma contamination, it is necessary to strictly follow the protocol below:

- Immediately notify Facility Staff.

- Isolate affected cultures for quarantine when disposal is not possible.
- Decontaminate all affected equipment and surfaces.
- Dispose of contaminated cultures according to biosafety procedures.
- Clean and disinfect the hood used, including UV sterilization for at least 30 minutes.
- Inspect all reagents and consumables potentially involved.
- Perform incubator cleaning and sterilization if required, according to the Facility Staff.
- Follow any additional instructions provided by the Facility Staff.
- Access restrictions may be imposed until decontamination procedures are completed.

Costs

Updates fees are available on the DSB Facility website.

Users will receive a biannual report that summarizes your reservations, based on the online booking calendar.