

LABORATORY INSTRUCTIONS, ROOM 4220**UiO : Department of Biology**
University of Oslo

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Building	Room number	Name of laboratory	Telephone
Kristine Bonnevie hus, BL18	4220	Laboratory for culture studies of eukaryotic microorganisms and cells	
Unit:	MERG, Department of Biology		

Key contact details

Role	Who	Phone	Office
Laboratory responsible	Kathrine Schou Cecilie Mathiesen	228 55196	4210
Training responsible	Cecilie Mathiesen	228 55196	4210
Safety representative	Cecilie Mathiesen	228 55196	4210
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Risk assessment summary**Biological Hazard**

Due to handling of unknown microorganisms or microorganisms with unknown toxicity this lab is classified as Biosafety level 2 (BSL-2). This is to ensure the health and safety of all users. This means that work performed has to comply with the Norwegian Regulation [FOR-1997-12-19-1322](#) (not available in English, but is in accordance with Directive [2000/54/EC](#)). Hence the risk assessment of this laboratory concludes that there is **MEDIUM** risk involved with use of this laboratory. However there are safety measures in place that limit the associated risk. It is very important to follow provided procedures and working guidelines. All users must act cautiously where and when is required.

BSL-2 differs from BSL-1 in that:

1. laboratory personnel have specific training in handling pathogenic agents and are directed by personnel with advanced training;
2. access to the laboratory is limited
3. extreme precautions are taken with contaminated sharp items; and
4. all procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets.



Activity

In this lab axenic eukaryotic cells and microorganism cultures will be established and maintained in growth chambers. Different types of growth experiments will be conducted. Many protist and nonfungal eukaryotes require highly specialized living conditions, hence the facility provide growth chambers that can be adjusted to simulate such environments by accommodate advanced daylight and temperature simulations. To facilitate for this diverse group of protist and non-fungal eukaryotes the laboratory is furnished with three BSC class II to prevent contamination.

- Growth of cultures of eukaryotic cells and microorganisms under various temperature conditions and CO₂.
- Experimental work on cultures of eukaryotic cells and microorganisms.
- Microscopy of cultures of eukaryotic cells and microorganisms.
- Storage of cultures of eukaryotic cells and microorganisms.
- Sampling of cultures of eukaryotic cells and microorganisms for further investigation e.g. RNA/DNA/protein analysis.
- Harvesting
- Extraction of microorganisms and cells from organisms or organs should **NOT** be performed in this laboratory. There are special facilities for such activities in other laboratories e.g. Grovlab MERG 4301, GrovLab CEES, 4436 or other suited facility.

General rules for all laboratories at the department:

- The HSE handbook applies
- Lab coats and gloves should be used at all times
- Work according to issued SOP including a step-by-step risk assessment.
- If an issued SOP is not present, a risk assessment has to be performed and documented (i.e. lab journal) before work commences.

General rules for all laboratories at MERG:**Preparation before starting work - training and planning:**

- All new users must take part in an introduction round arranged by room responsible before getting access.
- Documented training in use of Biological Safety cabinets-Class II and fume hoods is mandatory for all new users.
- All users must be approved before working in this laboratory. (See Appendix 3)
- New chemicals should be approved by lab responsible, who will ensure that the chemical is registered in ECOonline. The chemical has to be risk assessed in ECOonline. The chemical risk appendix 2 must be updated. New procedure including the new chemical must have a step-by-step risk assessment before using the new chemical in the lab.
- Always ensure that new chemicals including solutions are provided with a MSDSs.
- To make the laboratories easily accessible all key equipment has a booking log. Please check the booking log before using an instrument.

When working in the laboratory:**Handling of chemicals:**

- All chemicals should be stored in the Media laboratory; except microscopy chemicals, which will be stored in the Microscopy lab. Working solutions in daily use will be stored in the various labs.
- A copy of all MSDS / "Sikkerhetsdatablad" will be present in the labs where the chemical is being used and stored. This also applies to kit MSDS.
- All "homemade" working solutions should have a batch ID. The batch records are kept in the Media or Microscopy laboratory. Solutions should be labelled with Name of solution (e.g. 2X CTAB), Batch ID (Axxx), Data, Expiry date, Initials (who made the solutions).

Working according to the class II classification of the lab:

- To minimise known and unknown health risks associated working with microorganisms please always work in the Biological Safety cabinets-Class II.
- To prevent cross-contamination between cultures please always work in the Biological Safety cabinets-Class II. Even when working with known, non-hazardous organisms.
- There are provided designated lab coats in each room. Make sure that lab coats are left in the lab before you leave the room.
- Biological Safety cabinets-Class II and Fume hoods should be cleaned IMMEDIATELY after use.
- All spaces are shared between users. Please make sure that the workbenches are free from clutter. The workbenches are not to be used as storage space.
- Preparation of samples for DNA isolation should be performed in fume hoods or Biological Safety cabinets-Class II.
- Label your belonging with name or projects. All labelled items are private.
- Do not move laboratory equipment from or between rooms.
- **Hands MUST be washed in designated sink before leaving the room.**

Risk limitation

- All new protocols must be risk assessed and approved by laboratory responsible before work commences. Special precautions have to be made to prevent contamination and to ensure a safe working environment for people, samples and scientific experiments.
- Risk assessment of laboratory is to be performed annually or if new procedures and chemicals make this necessary. Please see appendix.

Specific rules: 4220 Laboratory for culture studies of eukaryotic microorganisms and cells

- As a precaution to prevent cross contamination working in more than one "cleanroom" per day is prohibited. 4220 Laboratory for culture studies of protists and other eukaryotes, 4224 Laboratory for culture studies of fungi and 4229 Laboratory for culture studies of prokaryotes
- As a precaution to prevent cross contamination from 4301 Laboratory for studies of "field material" working in 4220 Laboratory for culture studies of protists and other eukaryotes or 4229 Laboratory for culture studies of prokaryotes in the same day as working in 4301 Laboratory for studies of "field material" is prohibited.
- BEFORE using the centrifuge you need to be trained.
- BEFORE using the CO₂ incubator you need to be trained.
- BEFORE using the microscopes you need to be trained.
- Change shoes before entering the room

Health and safety equipment

- Nitrile and latex gloves
- Lab coats
- First aid kit
- Safety goggles in first aid cabinet
- Eye wash
- MSDS for all chemicals
- Folder with relevant HSE documentation (HSE handbook, SOP's)
- PigHaz™ mats for chemical absorption
- Shower head is present on main taps.

Waste disposal**Organisms:**

- Agar plates should be disposed in Hazardous waste receptacles/boxes for incineration.
- Hazardous waste receptacles/boxes for used tips, tubes etc.
- For adherent cells use VacuumSAFE, the medium should be autoclave before disposal.

Material:

- Glass receptacles for broken glass

Chemical:

- Please dispose of your surplus waste in correct container labelled as follows;
 - **Hydrocarbons** (unsubstituted, such as toluene, xylene, benzene, paraffin and cyclohexane):
 - **Halogens** (carbon tetrachloride, chloroform, methylene chloride, trichlorethylene, nitrobenzene, chlorobenzene).
 - **Alcohols, ketones and other water-soluble agents** (acetone, methanol, acetonitrile, dimethyl formamide, dimethyl sulfoxide, dioxane). Waste flasks must be clearly labelled to indicate the department/building and the contents.
- PigHaz™ mats are disposed in hazardous waste receptacle (after vapourisation is complete)

Instruments/equipment overview			
Name/type Serial number	Purchased year and supplier	Service agreement / Calibration frequency	Responsible
EnergySaver fume hood 09360-15452730	2011, Labflex	Annually, next time:	
Incubator, Thermax 10-342	2010, VWR	None required. A thermometer should be present in each incubator	
Incubator, Thermax 10-348	2010, VWR		
Incubator, Thermax 10-345	2010, VWR		
Incubator, Thermax 10-347	2010, VWR		
CO2 inkubator med kjøling og risting, INFORS S-000116593	2011, VWR	?	
Flameboy None	2010, Fischer	None required	
Fireboy 101039	2010, Fischer	None required	
Fireboy 101042	2010, Fischer	None required	
IKA ristebord, KS260B 06.024955	2010, VWR	None required	
IKA ristebord, KS260B 06.025282	2010, VWR		
Heraeus Multifuge X3R 41173167	2010, VWR	?	
Biological Safety Cabinets Cl. II, ESCO 2010-52626	2010, MEDINOR	10-11/5-2011 Next time: May 2012	
Biological Safety Cabinets Cl. II, ESCO 2010-54084	2010, MEDINOR		
Biological Safety Cabinets Cl. II, ESCO 2010-54085	2010, MEDINOR		
Vacuum pump 33862108	2010, VWR	None required	
Inverted microscope			
CEES microscope	Tilhører CEES		

Fridge- 09277892201112	2010, Expert		
Freezer – 9221831250331	2010, Expert		
Vortex, Serial # 0803 166 63	2008, VWR		
Eppendorf-pipette, 5000 Serial# 4422454	VWR	Last calibrated apr 2010, next calibration summer 2012	
Eppendorf-pipette, 1000 Serial# 2366258	VWR	Last calibrated apr 2010, next calibration summer 2012	
Eppendorf-pipette, 200 Serial# 3033703	VWR	Last calibrated apr 2010, next calibration summer 2012	
Eppendorf-pipette, 100 Serial# 1813715	VWR	Last calibrated apr 2010, next calibration summer 2012	
Eppendorf-pipette, 10 Serial# 2387468	VWR	Last calibrated apr 2010, next calibration summer 2012	
Eppendorf-pipette, 100-8channel Serial# 3468878	VWR	Last calibrated apr 2010, next calibration summer 2012	

Attachments:	
1. Risk assessment (1 page) 2. ECOonline chemical overview (4 pages) 3. Approved Users (1 page)	