**Lab rules for MIKPHYS Labs**

**Rooms: 3.108, 3.110 and 3.113**

**Department of Biotechnology and Food Science**

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# Rules for room 3.108, 3.110 and 3.113

Welcome to the labs 3.108, 3.110 and 3.113 in chemistry building 3. If you work at other labs at the department, you will find another set of rules for these labs. Ask your supervisor or some of the technical staff.

We expect you to follow the rules and hope you will like it here!

## Introductory comments

Plan your activities in the lab well. If you plan activities that can in any way harm yourself or your surroundings, you must document your risk assessment. Talk to your supervisor about this.

* Be careful with all activities in the lab. Even activities that seem trivial. Examples are heating media and hot water baths. Avoid placing very hot bottles into a cold environment. Do not eat or drink anything in the lab! We expect students to use common sense and be responsible.
* Please report any instrument errors to technical staff.
* Use googles, gloves, lab coat and shoe protection when required (e.g., filtering using vacuum/work with acids and bases). Use gloves that protect against the chemical you are working with (made of the correct materials). Safety data sheets contains this information.
* All toxic chemicals should be kept in a locked cabinet (cabinet or cooler) when not in use.
* Toxic chemicals in powder form should be handled (weight out) in the fume hood.

Important: For safety and practical reasons all chemicals are catalogued in the eco-online chemical library. Always put chemicals back in place after use. If you bring chemicals into the lab from another lab, and want to store it over time, please inform technical staff and we can register it in eco-online (or move it in the register).

## Making solutions

* Always clean scales/analytical balance after use (brush can be used to remove powder from enclosure on the analytical balance). Scales can be cleaned with wet paper towel or cloth.
* Always clean up any mess made on the benches (powder or solution).
* Glass- and plasticware used for making solutions, should be rinsed in water, and then placed in one of the dishwashers. Remove stickers/tape before dishwashing to avoid clogging the machines. If the dishwashers are in use, the rinsed equipment can be left on the sink next to the dishwashers. Dishwashers should be emptied by the first person to come by after a run is finished. Equipment left on the sink, should be included by the next person to run a dishwasher.
* Used spatulas can be placed in the beaker with water next to scales.
* Remove magnets from flasks with big magnet on bench (brown piece of metal). Do not leave magnets in flasks for long-term storage.
* Turn off and clean magnetic stirrers (remember to turn off heating if used).
* Do not contaminate stock chemicals. Never return chemicals to the stock containers and always use clean spatulas. For liquids: use a small beaker to measure approx. amount and then pipette from this (do not return remaining liquid to the stock chemical). Do not stick pipettes in stock chemicals.
* It’s everybody’s responsibility to order common chemicals and equipment before we run out. Inform the lab coordinator as soon as possible if we are running out of something (e-mail).
* Confer with the lab coordinator before you borrow/ remove chemicals from the room.
* Put chemicals back in the correct location (i.e., the location given in the substance index). If placed in the wrong location, other people will not be able to find it when searching the substance index.
* All solutions should be labelled according to IBT’s labelling routines (<https://i.ntnu.no/wiki/-/wiki/Norsk/HSE+at+IBT>).
* If hazardous chemicals are used in the production, the solutions should be labelled with the correct CLP pictogram.

## Genetically modified organisms (GMM)

The laboratories are approved for contained use of genetically modified microorganisms (GMM) in infection risk class 1. From this approval follows:

* 1. Wearing a lab coat is mandatory in laboratories. It is not allowed to enter the labs without wearing a lab coat (not even to fetch something/ask for someone). Guests to the labs can borrow a lab coat placed just inside the door at lab 3.113 and 3.110.
  2. GMM waste (including cells, antibiotics, and recombinant DNA) should always be inactivated (see below).
* Always turn off propane burners when not in use
* Clean your hands properly after any contact with chemicals or contaminated material (biological/ chemical).
* Boxes containing powder of antibiotics or toxic chemicals must only be opened inside ventilation hoods. Add solvent to obtain desired concentration of chemical inside a ventilation hood.
* Ethanol bottles should be placed in the ventilation hood when not in use. It is due to the fire safety rules. Maximum two 1-liter bottles with 96% ethanol are allowed to be in the ventilation hoods in one lab. If the bottles are empty, contact the technical staff for supply.
* Lab coats made of cotton must be autoclaved before they are washed. Put them in the big white nalge-box placed together with waste at room 3.110. Synthetic lab coats should not be autoclaved and must be delivered in the basement (level U2) in Chemistry building 3 (or contact Siri Stavrum).
* Areas contaminated with microorganisms must be disinfected with appropriate method. 70% ethanol is effective against many organisms.

### Autoclaving of GMM and general autoclave routine

* Mark the things to be autoclaved with content, date, name, and autoclave tape.
* Do not overfill bottles; they may boil and leak into the autoclave. Agar media and soya flour containing media should make up maximum 50 % and 20 % of the bottles total volume, respectively.
* Never tighten the screwcap so that vapor cannot escape – bottles may explode or the screw-cap may be damaged due to increased pressure.
* Solutions and items for autoclaving are put into the white box labeled “Not autoclaved yet” when the autoclave is in use (or you have only a few things). Be sure that you choose the right program when starting the autoclave. Check the water level of the autoclave chamber and that there is not too much water in the container for condensed water.
* Equipment that has been autoclaved is put in the blue boxes marked ”Autoclaved” and is collected by the owners as soon as possible. When you remove bottles from the autoclave, tighten the screw cap to avoid contamination from the air. All media containing agar should be placed in the heating cabinet under the bench.
* Waste that contains organic solutions (for instance DMSO) must not be autoclaved.

### Transport of GMM outside area approved for use

Transport of GMO (plates with bacteria, bacterial cultures, -waste) outside the approved area, should be done without risk of spill. For safe transport between laboratories, several methods for transport have been established:

1) Transport containers with lid (to be closed)

2) Zip-lock bags (closed)

3) Lab carts with high edges for transport of closed systems (buckets for autoclaving, bottles etc.)

In case of accidental spill outside the laboratories, the IBT emergency plan for spill of GMO should be followed.

## How to handle waste and perform cleaning?

### Inactivation of GMM waste

GMM-waste is inactivated by autoclaving. If the autoclaved liquid waste smells bad, make sure to empty the containers in the sink in a fume hood. “Naked” recombinant DNA and antibiotic solutions are also inactivated by autoclaving. Don’t throw this in ordinary waste!

Liquid microorganism waste, including GMM, that cannot be autoclaved, must be inactivated chemically, normally by addition of NaOH to > 0.5 M, incubation > 24 h. If this protocol is not suitable (e.g. spores), the project must identify satisfactory protocols.

### Plastic collection tank for liquid microorganism waste

Liquid microorganism-waste (e.g., cultures), including GMM can be disposed in a plastic tank/bottle in the fume hood at room 3.110. Lab users must autoclave liquid waste (1h, 120°C) when the tank is maximum half full. The tank (and its content) is cooled down to room temperature before being emptied into the sink. Use a sieve to collect any toothpicks, pipette tips and glass beads present in the container. Glass containers should be used for collection of cultures for chemical inactivation.

### Reusable equipment contaminated with microorganisms

Centrifugation tubes/flasks should be autoclaved with the lid taken off. Shake flasks can be autoclaved with cotton/silicone stoppers on. Do not put broken glass in the bucket. Contact lab engineer if you have contaminated broken glass/damaged glassware.

### Single time use equipment contaminated with microorganisms

Solid bacteria waste (plates) and plastic equipment for single time use (pipettes, tips, tubes, gloves etc.) that have been contaminated with bacteria must be discarded in the big, plastic buckets. Buckets must be supplied with an autoclaving bag before use. Do not fill the bucket too full before autoclaving (lid should fit).

Lab users are responsible for autoclaving the buckets. Ask your supervisor for more information on the washing routines in your lab.

### Ordinary waste and cleaning equipment not contaminated with bacteria

Paper waste and other non-contaminated waste is put into the ordinary waste bags. Smelly waste is put in sealed bags.

Uncontaminated broken glass/damaged glassware must be discarded into designated cardboard box with black plastic bag.

We use the washing machine in 3.117 since we do not have a machine in 3.113. **Talk to technical staff or supervisor to get info about the current routines.** In general, the dirty equipment is collected by the big sink in room 3.113 and brought to 3.117 for washing. Clean equipment is brought back in a blue container and placed into shelfs when it is dry.

**Some information about using the dishwasher:**

Placing equipment:When loading the dishwasher, try to place equipment on injector nozzles that fit the length of the equipment. If glassware is left dangling from a too long injector nozzle, equipment could potentially be damaged or break during dishwashing. Glass beakers should be placed on a minimum of two injector nozzles to avoid breakage. Do not place any equipment between nozzles. Rinse equipment before placing it in the dishwasher (corrosive chemicals and salts can cause damage to the machine). Be careful to remove any pipette tips, toothpicks etc. as such items may damage the machines or get stuck somewhere. Bottle caps, silicone closures, spatulas, stirring magnets and other small objects are placed in the separate dishwashing unit placed to the side of the sink. Small glass beakers and small glass funnels should also be placed in the separate unit together with the caps etc.

Cotton stoppers: Cotton stoppers cannot be washed in the dishwasher (will dissolve like paper and clog the filter). After autoclaving, cotton stoppers are simply put in the steel wire baskets on the bench for re-use. If a cotton stopper should happen to end up in a dishwasher, anyway, please remove it as soon as this is discovered and clean the filter (otherwise the dishwasher will be ruined). Contact the lab engineer if you need help.

How to start the dishwasher:open the dishwasher door and check the sieve combination at the inside bottom of the dishwasher. Rinse and clean the sieves if necessary and put them back. Measure out one measuring spoon of dishwashing powder and place in the powder chamber on the inside of the door. Close the powder chamber. The dishwashing powder, Deconex, is found in a box placed on top of the dishwasher (measuring spoon inside box). Check that nothing is blocking the nozzle that will flush the powder chamber (one, special nozzle placed in front of the machine, which is bent at the tip). Check that there is rinsing agent left in the bottles placed on top of the dishwasher.

### Organic solvents and toxic waste

Tanks (solvent bottles) are placed in the ventilation hood in 3.110 for disposing organic solvents (e.g. chloroform, phenol, DMSO). Separate between organic chemicals with and without halogens (fluorine, chlorine, bromine, iodine, astatine). If you work with other toxic chemicals and you do not find a category that these fits into, please talk to your supervisor or technical staff.

## Equipment in the labs

Spectrophotometer:

make an appointment if there is a queue, if it less than 2h until the next user – let it be turned on. Remember to use the logbook.

Working in the fume hood:

all work with solvents (also acetic acid) should be performed in the fume hood. The fume hood hatch should be closed after use.

Pressurized air:

Watch out for over pressure in glassware. Close tap when not in use!

Water bath:

Should be filled with distilled water.

Centrifuge:

Use the logbook if this is established, instructions can be found by the instrument.

pH-meter:

Use logbook. Check calibration. For reliable measurements the pH meter should be calibrated every day. Googles are mandatory (also valid when working with organic solvents).

Fume hood:

Avoid storing equipment and chemicals in the fume hood (exceptions: equipment that clearly belongs here such as pipettes, rack for tubes used for derivatization etc.). In case of power failure fume hood stops working. If this happens, stop all activity in fume hood.

Incubator with shaking:

Turn off, when not in use.

Gas flasks and regulators:

Do not change gas flasks or do any kind of handling of these without proper training.

Pipettes:

Pipettes are shared between users in the lab. Make sure your pipette is properly calibrated (pipettes in racks on the benches should be calibrated and marked).

Glassware found in room 3.108, 3.110 and 3.113 should not be mixed with glassware and equipment found in other labs. This has to do with cleaning and the fact that some of it is bought on different projects.

## Use of biological safety benches

* Before use: open front shield to working position and wait until air flow stabilizes before starting work in the bench.
* During work, try not to block air circulation at the ventilation slots of the work plate.
* Make sure that all items brought into the bench are as clean as possible.
* After finishing work: remove all your belongings from the bench and wash the working area with plentiful 70% ethanol. Wipe off with paper.
* Lift the bench plates to check if you have spilt something underneath and clean if so. Be sure to remove any pipette tips, glass beads or toothpicks that you may have dropped on the working area or below the bench plates.
* After cleaning: Close front shield and turn off the light. You may start the UV light for extra decontamination.
* OBS! The biological safety benches are not connected to the ventilation system, so do not use them for work with hazardous chemicals (use fume hood).

## Use of fume hoods

* Fume hoods should be used when working with hazardous chemicals (e.g., organic solvents, concentrated acids/bases, toxic, carcinogenic, or mutagenic chemicals) and antibiotics in powder form. This applies also when weighing out.
* Fume hoods should also be used when working with something with strong/unpleasant smell (even if not necessarily hazardous).
* Safety data sheets of chemicals or risk assessments must be checked to determine whether work can be on the bench or should be performed in a fume hood.
* When not in use, the front shield of the fume hood should be at a low position, as the fume hood will consume a lot of energy if left open.
* During work, the front shield of the fume hood can be raised to a higher position (max. level is indicated on the fume hood).
* After work in the fume hood: remove all your belongings, discard any waste and clean with paper towel. Be careful to remove any spill and do not leave used pipettes, tips, glass beads etc. behind as this can represent danger to other people in the lab.
* In the case that the fume hood is malfunctioning, or air flow is not sufficient: do not use the fume hood for work with hazardous chemicals.

## Equipment that requires special training

Freeze dryer:

Training is performed by technical staff.

Speedvaccum concentrator (placed in room U.15):

Training is performed by technical staff or an experienced user.

Waste from speedvac is emptied in a flask placed in the fume hood. If you have waste that fit in the category indicated on the bottle(s), establish a new bottle, or contact technical staff. Even if the lid can be opened, wait until the rotor stops before removing.

Autoclave:

Training is performed by technical staff or experienced user.

Bioreactors (Eppendorf BioFlo 115/320):

Technical staff, or an experienced user, performs training.

Bookitlab is used for reservation of most equipment that require training. Equipment that requires booking is marked.

**Contact your supervisor or technical staff if you have questions regarding equipment in the lab.**

## Order and tidiness

Each person is responsible for keeping both their own working space and used common working areas tidy and clean.

**Please ask more experienced staff if you are uncertain of how things should be handled in the lab!**

## Accidents in the lab

* Before starting up lab work, be sure to make yourself familiar with the emergency equipment available in the lab. Knowing what equipment is available and where you can find it, will make the response time much shorter in the case of emergency.
* Emergency showers are available in rooms 3.110 and 3.113. The showers are positioned over some of the doors (make sure you know where they are). The emergency showers are operated by pulling on the chain attached to it.
* Eye showers are available on many of the sinks. The eye showers can be pulled up from the sink to be held close to the eyes. You need to press a handle on the side of the eye shower to start the water flowing. There are also small eye shower flasks available. These can be brought along in the case that you need to leave the lab but need to continue rinsing. The small flasks contain a liquid that can neutralize a solution (acid or base), while the big flasks contain saltwater.
* Fire blankets are available in most rooms. Fire blankets can be used to put out small fires. Remember to leave the blanket on for some time after putting out a fire (let it cool down). Removing it too soon, can cause ignition of the fire again due to the high temperature.
* Fire extinguishers are available in most rooms. To use the fire extinguisher, remove it from the holder, pull out the safety pin, direct the hose at the fire and squeeze the handle. All fire extinguishers are with CO2 gas and can thus be used without concern for ruining lab equipment.
* Cabinets with first aid kits are available in most rooms. The cabinets contain different types of wound dressings like plaster, bandages, gauze and compress, CPR mask, scissors etc. If you use anything from a cabinet, notify the lab engineer so that the cabinet can be refilled.
* Whenever there is an accident resulting in damage/harm to humans, the environment, buildings or property, you need to report it through NTNU’s system for reporting problems and discrepancies: [RiskManager - Avvik (ntnu.no)](https://avvik.ntnu.no/Incident/Start). Hazardous situations or near-accidents should also be reported, as well as violations of routines, procedures or legislation and violence/threats. You can also register suggestions on how to improve health, safety, and environment (HSE) at NTNU. If you need assistance with a registration, contact the lab engineer for help.

## End of project/engagement/stay

As your project/engagement comes to an end, please make sure to:

* Clean up your lab space (empty cupboards and drawers and wash cupboard/drawers).
* Discard your solutions and return glassware to the dishwashers.
* Discard old samples, petri dishes etc. stored in fridges, cold room (+4°C), freezers (-20°C and -80°C) and cold storage (-20°C and -40°C). If you think something should be kept/stored discuss with your supervisor.
* Make a list of samples/strains left behind (including where it is stored) and send to your supervisor (not always relevant, please talk to your supervisor).
* Autoclave your lab coat(s).

## Checklist ‘guided tour’ in labs

* Introduce staff and users of the labs.
* GMO-lab. Require lab coat that must be left in the lab. New lab coats can be found in K3 U2.
* Gloves. Usually, nitrile. Do not leave the lab with gloves on! Doing so is a bad habit and may cause cross contamination. Even clean gloves can rise concerns among your co-workers.
* Chemicals. Where? Do not move chemicals “permanently”, or bring new chemicals into the lab, without notifying the person responsible for the lab. Any changes must be registered in eco-online. If not, eco-archive will soon be outdated.
* How to place chemicals? Generally, place chemicals with similar properties grouped together.
* Stock solutions and chemicals in non-original bottles or boxes must be labelled with appropriate chemical hazard symbols, initials, date, content, and concentration/purity.
* Familiar with eco-online? Need to know how to find chemicals and their chemical safety data sheet (SDS).
* Use of nitrogen gas. Close the tap when finished! Nitrogen come from central gas storage in K5 (expensive). Let person on room card know if high consumption is planned.
* Use of gases. Do not change regulator without proper training. Notify the person on room card when replacing/removing/importing flasks into the room.
* Lab spaces? What belongs to who?
* Fume hood (correct use). Close when not in use. Operate at correct working height. Remove things that do not need to be stored in the fume hood after your working session.
* Ordering. General rule is that all persons can order (except students). Write what is ordered on the list hanging on lab door. When consumables are empty (ideally before), remember to order new.
* Explain how to deal with chemical waste. Glass, toxic and chemical spill. Empty chemical boxes. Make sure the boxes are totally empty and cross out the chemical hazard symbols before placing them in the box by the “waste-fume hood”.
* How to deal with chemical spill? Contact person on room card. What can you deal with yourself?
* Instrumentation. What equipment require training? (E.g., bioreactor, freeze dryer and speedvac).
* Booking system for instruments. E.g., plate reader, speedvac, freeze dryer, sampling equipment, bioreactors etc.
* Keep the lab tidy! When you finish your work in the lab (especially for students) make sure all stock solutions etc. are emptied and everything that is not passed on to another person is thrown away.
* Washing of glass equipment. How and where?
* When and where to use lab googles?
* What to do in case of fire? Give the location of the nearest fire extinguisher and fire blanket. (Refer to NTNU’s fire regulation. Small fires should be put out. Larger fires and/or fires that involve larger amounts of solvents, trigger the fire alarm, notify, and evacuate).
* Risk assessment must be updated when starting new lab activities. Are you familiar with the risk assessment form?