

Laboratory Safety Guidelines

ETH alarm centre (24h service):
from internal phones: 888
from external/cell phones: +41 44 342 1188

General Laboratory Practice at Biosafety Level 1 (BSL1)

Professional standards of personal behavior in the laboratory ensure your and your coworker's safety. You are responsible that your work is safe to you, to others and to the environment. For more information on biosafety, please check also the ETH Biosafety Concept: https://ethz.ch/content/dam/ethz/associates/services/Service/sicherheit-gesundheit-umwelt/files/biosicherheit_neu/en/Biosafety_concept.pdf

- Do not consume/store food or beverages in the laboratory.
- Do not allow visitors, including children, in laboratories where hazardous substances are stored or are in use or hazardous activities are in progress.
- Do not wear open shoes or shorts in the lab.
- Lab coats are mandatory in BSL1 laboratories. Wear gloves and safety goggles when handling hazardous material. It is strongly recommended not to wear contact lenses, also not in combination with safety goggles.
- When leaving the lab, remove gloves and lab coats. No lab coats and gloves in offices, seminar rooms, toilets and kitchen! Outside the lab area, gloves have to be removed especially in elevators and when touching doors!
- Hands should be washed after handling biological material, after removing gloves and when leaving the laboratory.
- Do not pipette by mouth; only mechanical pipetting devices are permitted.
- Perform all procedures carefully to minimize the creation of splashes or aerosols.
- The laboratory should be kept clean and free of material not pertinent to the work. Work surfaces should be decontaminated at least once a day with 70% alcohol.
- Any spill has to be removed immediately. Put up warning signs if floor is wet.
- The use of needles and syringes should be restricted. When used, care must be taken to avoid injuries. Dispose needles, syringes, broken glassware and other sharp things into appropriate containers.
- Contaminated liquids or solid materials have to be decontaminated before disposal (procedure see below).
- Use a bottle carrier, cart or other secondary container when transporting chemicals in breakable containers (especially 250 ml or more) through hallways or between buildings. The individual transporting the chemical should know about the hazards of the chemical and should know how to handle a spill of the material.
- Use a basket or cart for transporting glass containers with liquids, culture medium or cell cultures within or between buildings.
- Know the location and how to use emergency equipment, including safety showers, eyewash stations and medical cabinets.

- Be alert to unsafe conditions/actions. Report them to your supervisor immediately.
- Any biological accident or emergency (definition see below) must be reported to the IMB biosafety officer Dr. Christoph Giese (giesec@mol.biol.ethz.ch, phone: 33292) or deputy biosafety officer Dr. Marc Leibundgut (leibundgut@mol.biol.ethz.ch, phone: 33148) and the project leader.
- Special safety considerations apply during pregnancy, which are not covered here. You are advised to contact any of our safety officers or your supervisor to discuss this individually. For questions about pregnancy and the evaluation of your safety at your work place please e-mail mutterschutz_cabs@ethz.ch as early as possible. You may also contact: Dr. Ines Raabe (chemistry, biosafety, lab safety, ines-raabe@ethz.ch, phone: 26809) or Dr. Silke Kieseewetter (work safety, radiation protection, lab safety, silke-kieseewetter@ethz.ch, phone: 27629). Your concern will be treated confidentially. Detailed information about maternity protection can be found here: https://ethz.ch/content/dam/ethz/associates/services/Service/sicherheit-gesundheit-umwelt/files/mutterschutz/en/Maternity_protection-factsheet.pdf

Organization of Biological Safety

For the permission to work with biological material, we have to follow ETH, Cantonal and Federal regulations. The entire institute is certified for BSL1 work, while BSL2 work is restricted to specifically equipped rooms. Although none of the BSL1 organisms you are working with is considered dangerous, several rules have to be followed:

- Reporting of novel activities or significant changes in ongoing projects to the biosafety officer (Dr. Christoph Giese, giesec@mol.biol.ethz.ch, phone: 33292) is compulsory. This includes the work with organisms/strains (viruses, bacteria, fungi, cell cultures...) as well as recombinant work with toxins or pathogenicity factors.
- A risk assessment has to be done in consultation with the biosafety officer *before* the start of an activity. For this, both Federal (BAFU) and BSL classifications of commercial strain collections (ATCC and DMSZ) have to be taken into account.
- Proper documentation of any work with biological material is mandatory and has to be available at any time.
- Special rules apply for BSL2 work. You must receive additional instructions in order to be allowed to work in a BSL2 area. Contact the biosafety officer (Dr. Christoph Giese, giesec@mol.biol.ethz.ch, phone: 33292) before you start working.

Biological Accident or Emergency

What is a biological incident?

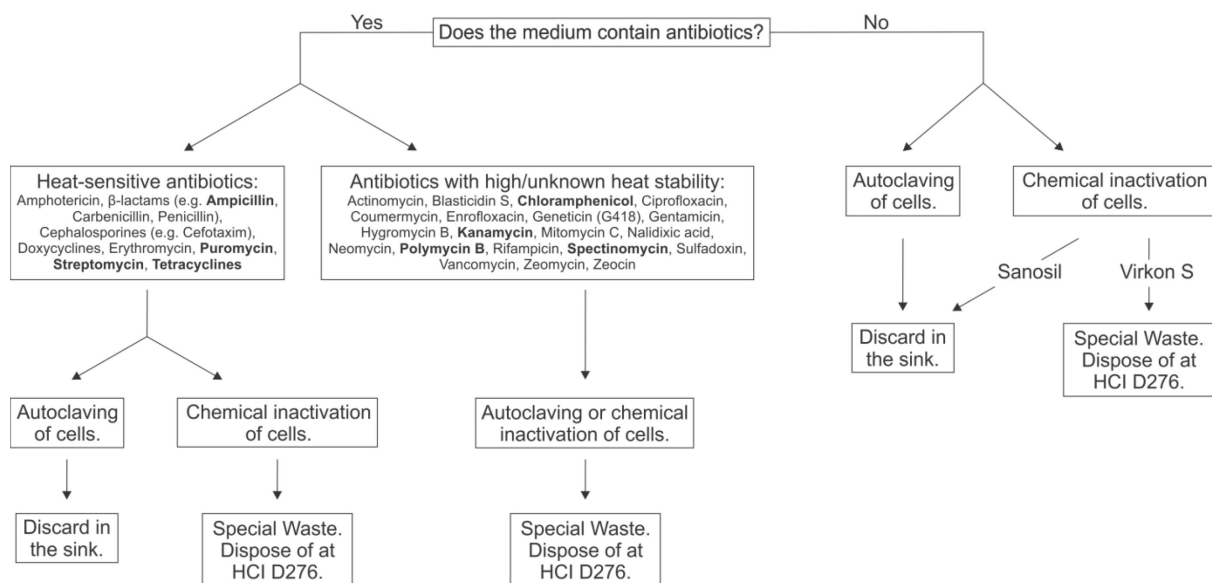
- Bacterial/Cell culture splatters in the face
- Injury with contaminated sharp items (broken glassware, syringe...)
- Spillage of large volumes (>1L) of cell culture
- Uncontained cell culture loss (>100ml)

What to do:

- Skin/face contact: Wash face extensively with water, at many sinks an eye shower is installed (flush tubing for a moment before use)
- Injury: Wash with water, consult medical doctor (provide detailed info yourself if possible)
- Spill: Use the “Spill kit” available in each lab. Clean up with paper towels (to be autoclaved prior to disposal), decontaminate with 70% alcohol or bleach (careful, your clothes will be destained and corroded in case of contact).

Biological Waste and its Disposal

- For intermediate storage, waste must be collected in designated places labeled “Solid biological waste” or “Liquid biological waste”.
- Solid waste: Agar or any solid material that was in contact with cells (petri dishes, pipette tips, toothpicks, cuvettes, soaked paper towels...) Solid waste has to be autoclaved in special biohazard bags at 121°C for 20 min.
- Liquid waste: Cultures and culture supernatants have to be either disinfected with sodium hypochlorite (bleach) solution (f.c. 1-5% for >10min) or equivalent (e.g. Sanosil © according to manual), or autoclaved at 121°C for 20 min. Depending on the method of inactivation used and whether hazardous chemicals (e.g. antibiotics) are present in the inactivated liquid, the disposal is done according to this scheme:

Disposal of cell culture media

Disposal sites for special waste:

HCl D276

Tue, Thu & Fri 1-4 pm (during semester)

Tue & Thu 2-4 pm (semester break)

HPL D15.2

1st Tue each month 9-11 am

for on-demand appointments contact:

sgu-sonderabfall@ethz.ch

Chemical Safety

Many lab accidents happen due to inappropriate/insufficient protective clothing. If you or people around you are handling strong corrosives (NaOH, KOH, acids, phenol, HCl, phosphoric/acetic acid) or liquid nitrogen/propane/ethane, make sure that you are wearing protective clothing and safety goggles.

Brief (incomplete) list of hazardous chemicals frequently encountered in biochemistry/structural biology laboratories (more information follows below the list):

- NaOH, KOH, HCl, phosphoric/acetic acid: (corrosive)
- Sulfuric acid: (toxic, corrosive, harmful to environment)
- Phenol: (toxic, corrosive)
- Uranyl acetate: (very toxic, radioactive, carcinogenic, harmful to environment)
- Hg compounds: (very toxic, harmful to environment)
- Ethidiumbromide: (very toxic, carcinogenic)
- Sodium azide: (very toxic, harmful to environment)
- Acrylamide: (toxic, neurotoxic, carcinogenic?)
- Ni, Co compounds: (toxic, allergenic, harmful to environment)
- Cycloheximide: (very toxic); Hygromycin: (very toxic); Ampicillin: (noxious) (all are harmful to environment)
- Liquid nitrogen: can cause strong cold burns upon prolonged contact with skin. Avoid splashes into eyes and soaking of clothes/cotton gloves!
- Liquid ethane: causes immediate strong cold burns on skin

For more detailed information (factsheets, concepts, guidelines) on specific hazardous substances, check the SGU website: <https://ethz.ch/staffnet/en/service/safety-security-health-environment/dokument-a-bis-z.html>

Before handling hazardous substances, always read the material safety data sheets (MSDS) and follow the instructions specified in there.

Attention: When handling hazardous substances (corrosives, toxics, flammables, etc.), work in a chemical fumehood and wear protective equipment (gloves, safety glasses, labcoat)!

Hazards to be aware of in biochemistry and structural biology laboratories:

Corrosives

Corrosive liquids (e.g. mineral acids, alkali solutions and some oxidizers) are significant hazards because skin or eye contact can readily occur from splashes and their effect on human tissue takes place very rapidly. The eyes are particularly vulnerable. Eye protection must be worn when corrosive chemicals are handled. Work in a fumehood when handling corrosives. Use labcoat, goggles and appropriate gloves.

When concentrated acids and bases are mixed with water, make sure to always add acids or bases to water (and not the reverse) to avoid a flash steam explosion due to the large amount of heat that is produced. Acids and bases have to be segregated for storage. Liquid corrosives should never be stored above eye level.

Flammable and combustible liquids

Flammable and combustible liquids vaporize and form flammable mixtures with air when in open containers, when leaks occur, or when heated. To control these potential hazards, several properties of these materials, such as volatility, flashpoint, flammable range and autoignition temperatures must be understood. Information can be found in that liquid's material safety data sheet (MSDS). Flammable and combustible liquids should be stored only in approved containers.

- Quantities should be limited to the amount necessary for the work in progress.
- Flammable liquids should be stored separately from strong oxidizers, shielded from direct sunlight, and away from heat sources.

Flammable liquids commonly used: Ethanol, Methanol, 2-Propanol, Acetone; in addition harmful to your health: Acetonitrile (for HPLC), Chloroform, Dimethylsulfide, Ethylacetat, n-Hexane, Toluol, Pyridine, tert-Butylalkohol, Diethylether (highly flammable). Work in a fumehood when handling flammables. Use labcoat, goggles and appropriate gloves.

Phenol

The major hazard of phenol is its ability to penetrate the skin rapidly, causing severe burns. Toxic and even fatal amounts of phenol can be absorbed through relatively small areas of skin. *In case of skin contact:* Remove contaminated clothing (protect yourself!) and immediately flush skin with large amounts of water. Flush skin with water for at least 30 minutes. Intermittently, and as soon as possible, apply polyethylene glycol (PEG 400 MW solution, as it does not enter skin) to affected area and then rinse off with water. Seek medical attention. *In case of eye contact:* May cause severe damage and possibly blindness. Immediately flush eyes with plenty of water for at least 15 minutes, lifting eyelids occasionally. Get medical attention immediately. Work in a fumehood when handling phenol. Use labcoat, goggles and appropriate gloves.

Heavy atom compounds

Heavy atom compounds commonly used for EM and derivatization of crystals (uranium, mercury etc.) are extremely toxic and carcinogenic and might in certain cases even penetrate rubber gloves. Therefore always wear the blue nitrile gloves when you handle such compounds. Never touch any door handles with contaminated gloves.

Ethidium bromide and other DNA-intercalating dyes

The intercalating DNA/RNA dye ethidium bromide (EtBr) used for visualization of DNA/RNA in agarose or acrylamide gels is extremely toxic and carcinogenic and penetrates rubber gloves. Therefore always wear the blue nitrile gloves when you handle this chemical. Never touch any door handles with contaminated gloves. SYBR® Safe stain is considered a somewhat less hazardous alternative to EtBr that can be used with either blue-light or UV excitation, but it nevertheless should be handled with the same caution, since it also intercalates DNA. Follow the rules described in the SGU factsheet on EtBr.

Sodium azide and other azide salts

In almost all crystallization solutions, sodium azide is added to prevent unwanted growth of microorganisms. Azides are acutely toxic since they irreversibly inhibit the respiratory chain

enzyme cytochrome-c oxidase. Symptoms are often compared with those of cyanide. Ingestion of dust or solutions can induce the following symptoms within minutes: rapid breathing, dizziness, weakness, headache, nausea and vomiting, rapid heart rate. Always wear gloves when pipetting screens. Heavy metal azides, particularly copper azide, are highly explosive and can detonate on touch. Keep them away from heat sources and avoid applying mechanical force on them (e.g. grinding in a mortar!)

Acrylamide and SDS

The acrylamide used for preparing polyacrylamide gels is toxic and potentially carcinogenic, mutagenic and toxic for reproduction. Avoid skin and eye contact, always wear gloves, lab coat and safety goggles. Handle acrylamide, and cast acrylamide gels, only in the fume hood. Store acrylamide in a ventilated cabinet. Solid SDS is a very fine powder that irritates the lungs and has been suggested to have similar effects (although less potent) as asbestos when inhaled. Work in the hood and wear a suitable mask (FFP3) when weighing out SDS and avoid spreading around.

Metal affinity purification

Nickel and cobalt used for affinity purification of proteins are toxic and allergenic. Use gloves.

Compressed gases

Compressed gases can be toxic, flammable, oxidizing, corrosive, inert or a combination of hazards. Use compressed gases only in areas that can be well ventilated. Follow the rules stated in the ETH Gas Guideline

(https://ethz.ch/content/dam/ethz/associates/services/Service/sicherheit-gesundheit-umwelt/files/chemikalien_gas_gefahrstoffe/en/Gas_Guidelines-gas_cylinders_with_compressed_and_liquefied_gases.pdf).

Flammable gases: Keep sources of ignition away from the cylinders.

Inert gases: Asphyxiation is a hazard associated with any gas, but particularly with *inert gases* (like nitrogen) when the gas is released rapidly and displaces air oxygen, e.g. through a broken regulator stem.

In addition to the chemical hazards, compressed gases are under high pressure. The amount of energy in a compressed gas cylinder makes it a potential rocket. A compressed gas cylinder will shoot through a concrete wall when the regulator stem is sheared off. Appropriate care in the handling and storage of compressed gas cylinders is essential.

Handling Precautions

- Avoid dropping, dragging or sliding cylinders. Use a suitable hand truck or cart equipped with a chain or belt for securing the cylinder to the cart, even for short distances.
- Cylinder caps should be left on each cylinder until it has been secured against a wall or bench or placed in a cylinder stand, and is ready for installation of the regulator. Cylinder caps protect the valve on top of the cylinder from damage if knocked.



- Toxic, flammable and corrosive gases should be carefully handled in a hood or gas cabinet. Proper containment systems should be used and minimum quantities of these products should be kept on-site.
- Never use the cylinder valve itself to control flow by adjusting the pressure.

Cryogenics

Cryogenic liquids have boiling points of less than -73°C (-100°F). Cold burns and frostbite caused by cryogenic liquids can result in extensive tissue damage. The cold boil-off vapor of cryogenic liquids rapidly freezes human tissue. Materials such as plastics and rubber become brittle and might fracture under stress at these temperatures.

Cryogenic liquids produce large volumes of gas when they vaporize. Liquid nitrogen will expand 696 times as it vaporizes. If these liquids vaporize in a sealed container, they can produce enormous pressures that could rupture the vessel. For this reason, pressurized cryogenic containers are usually protected with multiple pressure relief devices.

Follow the rules in the ETH Cryogenics Guideline

(https://ethz.ch/content/dam/ethz/associates/services/Service/sicherheit-gesundheit-umwelt/files/chemikalien_gase_gefahrstoffe/en/Gas_Guidelines-Cryo-Gases-use_of_cryogenic_liquefied_gases_and_dry_ice.pdf).

Vaporization of cryogenic in an enclosed area can cause asphyxiation.

Handling Precautions

- Always handle these liquids carefully to avoid skin burns and frostbite. Exposure that may be too brief to affect the skin of the face or hands may damage delicate tissues, such as the eyes.
- Boiling and splashing always occur when charging or filling a warm container with cryogenic liquid or when inserting objects into these liquids. Perform these tasks slowly to minimize boiling and splashing. Use tongs to withdraw objects immersed in a cryogenic liquid.
- Never touch uninsulated pipes or vessels containing cryogenic liquids. Flesh will stick to extremely cold materials. Even nonmetallic materials are dangerous to touch at low temperatures.
- Cylinders and dewars should not be filled to more than 80% of capacity, since expansion of gases during warming may cause excessive pressure buildup.
- Face shield or safety glasses should be worn during transfer and handling of cryogenic liquids.
- Wear dry, insulated cryogenic gloves when handling objects that come into contact with cryogenic liquids and vapor.
- When handling **flammable gasses in liquid form**, such as liquid propane or liquid ethane, keep sources of ignition away.

Dry Ice

- Wear insulated, dry gloves when handling dry ice.
- Do not lower your head into a dry ice chest, since suffocation can result from carbon dioxide buildup.

Sonicator

Wear ear protection during operation, use in a closed, sound-isolated containment.

Storage and disposal of chemicals

Organic solvents and acids must be stored separated from each other in ventilated cupboards or in fume hoods.

Chlorinated and non-chlorinated solvents must be collected separately in labeled containers. Very toxic chemicals (mercury, arsenic compounds, ethidium bromide etc.), acids, bases and heavy metals have to be collected in separate, properly labeled containers. Full containers are brought to the Special Waste Disposal Center in HCI D276. If you are uncertain how to dispose of your chemicals, contact the Special Waste Disposal Team via sgu-sonderabfall@ethz.ch.

Working Alone

When performing hazardous work, make sure that there is always a second person present in the same lab.

Laboratory Emergencies

Phone Numbers ETH Alarm Centre (24 hour service): 888
 (or from cell phone: 044 342 11 88)
 Fire department: 0-118
 Medical Emergency / Ambulance: 0-144
 Police: 0-117

Safety officers

Biological safety: Dr. Christoph Giese (giesec@mol.biol.ethz.ch, phone: 33292); Deputy: Dr. Marc Leibundgut (leibundgut@mol.biol.ethz.ch, phone: 33148)

Chemical safety: Dr. Christoph Giese (giesec@mol.biol.ethz.ch, phone: 33292); Deputy: Dr. Marc Leibundgut (leibundgut@mol.biol.ethz.ch, phone: 33148)

Radiation safety: Dr. Christoph Giese (giesec@mol.biol.ethz.ch, phone: 33292); Deputy: Nina Tremp (nina.tremp@mol.biol.ethz.ch, phone 36410)

Fire

- Remain calm.
- Call the ETH Alarm Centre (888) and fire department (0-118).
- **If the fire is large or spreading**, activate the fire alarm to alert building occupants. Inform persons at risk and, if possible, assist persons out of danger
- **If your clothing catches fire**, drop to the floor and roll to smother the fire. If a co-worker's clothing catches fire, get the person to the floor and roll him or her to smother the flames. Use a safety shower immediately thereafter.

- Close doors and windows.
- Quench fire.
- Fire extinguisher, emergency kit and important phone numbers can be found close to the stairways
- **Don't put yourself into danger!**

Chemical Emergency

In the case of chemical emergency inform the Chemical Safety Coordinator Dr. Christoph Giese (giesec@mol.biol.ethz.ch, phone: 33292) and the project leader.

Routes of contamination with chemicals: skin, eyes, inhalation, accidental injection

The simplest way for chemicals to enter the body is through direct contact with the skin or eyes.

Chemicals on Skin

The absorption of a chemical through intact skin is influenced by the health of the skin (cracks, cuts, injuries) and the properties of the chemical. Hydrophobic chemicals like organic solvents can easily penetrate skin.

- Immediately flush with water for no less than 15 minutes. For larger spills, the safety shower should be used. Remove contaminated clothing.
- Check the Material Safety Data Sheet to determine if any delayed effects should be expected.
- For phenol concentrations of more than 10%, flush with water for 15 minutes or until the affected area turns from white to pink. Apply a solution of 400 molecular weight polyethylene glycol.

Chemicals in Eyes

Chemical contact with eyes can result in painful injury or loss of sight. Wearing safety goggles reduces the risk of eye contact with chemicals.

- Immediately flush eye(s) with water for at least 15 minutes. The eyes must be forcibly held open to wash, and the eyeballs must be rotated so all surface area is rinsed. The use of an eye wash fountain is desirable so hands are free to hold the eyes open.
- Contact lenses should not be worn in the lab. In case injured person violated this rule: Remove contact lenses while rinsing. **Do not lose time removing contact lenses before rinsing.** Do not attempt to rinse and reinsert contact lenses.
- Seek medical attention regardless of the severity or apparent lack of severity.

Accidental Injection of Chemicals

This might happen when handling needles or sharps (scalpels, razor blades etc) or when contaminated glass breaks while being handled. Wash the area with soap and water and seek medical attention, if necessary.

Chemical Inhalation

The respiratory tract is the most common route of entry for gases, vapors, particles, and aerosols.

- Close containers, open windows or otherwise increase ventilation, and move to fresh air.

- If symptoms, such as headaches, nose or throat irritation, dizziness, or drowsiness persist, seek medical attention.

Guest scientists/external scientists at the Institute of Molecular Biology and Biophysics (IMBB)

It frequently occurs that scientists from other laboratories come to IMBB to process samples and/or record data on IMBB instruments. The respective contact person from IMBB has the responsibility to inform the guest/external scientists about IMBB's safety regulations. Specifically, guest/external scientists are only allowed to perform experiments at IMBB, when they have confirmed (with signature) that they have read and understood IMBB's safety regulations and will follow them. For this purpose, printed copies of the safety regulations are available in the IMBB Manager's office (HPK D19), as well as forms to be signed by guest/external scientists. In addition, guest/external scientists have to inform the IMBB Safety Officer or the respective IMBB professor about the identity of the samples they are planning to bring along to the Institute. Experiments with external samples may only be started i) after the samples have been approved by the respective IMBB professor or the IMBB Safety Officer and ii) provided that all experiments are in accordance with the safety regulations of the IMBB.

This handout only covers general safety guidelines. Before working with chemicals or equipment unfamiliar to you, consult the "Material Safety Data Sheets (MSDS)" and request training by a senior researcher authorized to run the equipment. For most institute instruments a responsible contact person has been assigned.

Hereby I confirm that I have read and understood these safety instructions.

Date

Name (printed)

Signature