Basic laboratory rules & safety constructions

ECUST_China IGEM 2019

Basic Laboratory rules and safety constructions

Laboratory is an important place for scientific research and learning. In order to create a clean and comfortable experimental environment for everyone, those who work and study in the laboratory must abide by the relevant regulations of our laboratory.

1. Basic rules

1.1 laboratory safety

Everyone should wear white coats and gloves at all times during conducting experiments, and wear protection glasses when necessary (e.g., using liquid nitrogen, sterilization pot, alcohol lamp, UV transmission systems (should wear UV protective glasses)), wearing masks (using volatile, respiratory stimulant regents, etc., at the same time performing in a ventilator).

Slippers, sandals and miniskirts are strictly prohibited. Do not eat or drink on the laboratory bench.

When leaving the laboratory and entering public places, take off your lab clothes and wash your hands. If it is necessary to use the elevator to transport the test items, take off your gloves before opening the door and press the elevator.

The last person who leaves the laboratory at any moment, even for a short time, needs to lock the iron door inside. The last person to leave at night or on weekends is responsible for the final check of the laboratory safety, including closing water, electricity, gas valves, and turning off air conditioning, sure that doors and windows locked. Turn off the power of water bath pot, circulating water bath, centrifuge and other apparatuses, but leave the lids of cryo centrifuges open.

Learn about emergency measures. Read the contents related to "laboratory security management" on Baidu Netdisk.

1.2 basic operation

Before using any reagents or drugs, one should thoroughly learn the physicochemical properties, operating environment, toxicity, and how to deal with poisoning, and how to deal with the waste liquid (read the chemical safety technical specification (MSDS) of the reagents).

It is strictly forbidden to use the same weighing spoon to take different drugs, or to retrieve the drugs back to the original bottle. And it is strictly forbidden to use molecular biological reagent without changing the tip, which may results in cross contamination.

Do not grasp any chemical reagent by bare hand, do not taste any reagent, do not use mouth to blow pipettes to take liquid.

Be familiar with the proper use of all instruments involved in the work, including safety precautions, take good care of public laboratory equipment, and strictly follow the operation rules. If there is any problem in the process of use, please inform the management for further treatment, and do not dismantle or repair without authorization. It is forbidden for new students to operate experimental instruments alone. They should use them under the guidance of senior students.

After using the instrument, the laboratory personnel shall be responsible for keeping the

instrument clean and pollution-free, and keeping the laboratory table clean and tidy.

The use of laboratory instruments should be booked and recorded before, during and after use. Anyone who needs to use instruments in the platform of State Key Lab should go to platform teacher for application forms, and make the instrument registration at the same time.

Pressure vessels must be trained to operate independently.

Check regularly whether there is enough water in the water bath pot to prevent dry burning and causing fire. The water bath pot need keeping powering off during holiday.

Do not leave people when using devices such as open flame (alcohol lamp), microwave oven and sterilizer.

Sample storage: mark the sample name, preparation time and owner's name, and put it in the sample box and the designated storage space of refrigerator and freezer. Do not put it in random places. Private samples are forbidden in the public reagent space. Unused public reagents (such as antibiotic reservoir, DTT, IPTG, etc.) should be put into their own sample boxes for further use next time, and should not be directly put back into the public kit.

Check the stock of samples and place orders in time.

For the preparation of common reagent, relevant personnel (first-year graduate student and undergraduate students of graduation program) should prepare the reagent according to the requirements in time to avoid delaying the experiment.

1.3 student ethics

Be inquisitive, communicate with seniors, consult literature and discuss with teachers before conducting new experiments.

Not absent from the laboratory during normal working hours and not absent for no reason. If you need to leave school, you should ask for leave in advance. If you leave school on weekends or long holidays, you should inform your tutor or classmates about your destination.

Make a good work plan and record experimental progress every week. During normal working hours, priority should be given to completing bench work (working on the laboratory bench), and literature reading should be conducted between experiments. Write a weekly electronic summary of the experiment and next week's work plan.

In normal working days (Monday to Friday), You should not engage in things unrelated to experiment and study in the laboratory (such as watching movies, variety shows, series, playing games, etc., except watching video and network lectures related to experiment).

It is forbidden to download and install games and entertainment software and store movies and TV series on the laboratory computer.

2. Experimental operation rules

2.1 microbial culture and operation

2.1.1 laboratory sterilization operation

In the laboratory, the culture medium used for bacterial culture and the centrifuge tube, EP tube

and the head of a pipette needed for the production of competent cells need to be used after high-temperature and high-pressure sterilization. Do not use the autoclave sterilizer alone without training. Precautions for use of sterilizer:

a. The state of sterilization pot must be checked before use--The needle of the pressure gauge must point to zero. Check whether the condensed water level is within its safety indication limit, and pour out some condensed water when it exceeds the upper limit of the indicated water level.

Check whether there is enough water at the bottom of the pot. It is required that the water at the bottom of the pot should be equal to the bottom of the basket below when sterilizing, and an appropriate amount of water should be added when it is not enough (100ml tap water and deionized water). After checking, power on and start up.

b. After starting, the indicator light will be on.

The main indicator panel shows 121℃, 20min. When the left panel displays Lid, it indicates that the Lid is uncovered. Display Bottle to indicate insufficient or excessive condensation. When Water is displayed, it indicates that there is not enough Water in the bottom of the pot, and Water needs to be replenished.

c. When opening or closing the lid, step on the pedal.

d. Place items

Items put into the pot for sterilization shall be affixed with high temperature and high pressure sterilization instructions.

Put into the solid for sterilization, do not put too much, especially do not touch the lid, to prevent the pressure cooker ventilation valve is blocked. When sterilizing centrifugal tube, blue-cap bottle, etc., it is required that the bottle cap cannot be tightly closed to prevent the high-pressure sterilization deformation of the tube body or bottle bursting.

When sterilizing liquid material, the -sterilize liquid must be selected in the sterilizing mode. The cap should not be tight and the liquid in the bottle should not exceed 2/3 of the volume. When removing the liquid medium in a triangular shaker, the appropriate volume of the medium should not exceed 1/4 of the bottle volume (1/5 of the bottle volume is recommended, i.e., 100ml liquid medium in a 500ml shaker).

Before sterilization, pay attention to whether sterilized items (especially containers with liquid) are placed firmly to prevent scattering and splashing.

e. After sterilization, open the lid only when the index of pressure gauge is 0.

When there is culture medium in the sterilizing pot, it is required to take out the culture medium in time (there is no need to wait for the temperature to drop to room temperature). It is forbidden to place the culture medium in the sterilizing pot for too long.

Remove items with insulated cotton gloves and chemical goggles to prevent burns or liquid spillage.

When sterilizing the waste plate, it is necessary to check whether there is any leakage after sterilizing, and clean up the leakage in time, and completely replace the water in the sterilizing pot.

2.1.2 microbial culture

Bacterial inoculation, plate marking, plate pouring and other operations require a sterile environment and must be carried out in a super clean platform.

2.1.2.1 specifications for the use of ultra-clean platform

a. Open the ultraviolet lamp for ultraviolet sterilization for half an hour before using the ultra-clean platform. Open the cabinet door before using and blow for several minutes until there is no ozone smell in the ultra-clean platform.

b. When inoculating bacteria, it is required to tilt the test tube so that the culture medium flows to the mouth of the test tube, add the bacteria liquid or antibiotics to the tube mouth culture medium (only the suction head contacts with the culture medium), and then put the test tube upright to make the mixture uniform. It is forbidden to insert the entire lower end of the piperator directly into the test tube to receive bacteria. Follow the same procedure for sampling. New students should ask senior students to demonstrate aseptic operation for the first time.

c. After the ultra-clean table is used, it is forbidden to pile up personal belongings. After the table is cleaned, wipe the table with 1% disinfectant (MCG) and 70% alcohol successively, and then close the ultra-clean table.

2.1.2.2 fell flat

a. An agitator should be added to sterilize the culture medium before sterilization. The sterilized culture medium is cooled to about 50℃ (just touch the bottle with the wrist, and you will not feel the heat), and corresponding antibiotics should be added to the resistant plate, and placed on the magnetic stirrer with low speed to stir evenly.

b. Turn on the alcohol lamp in the ultra-clean platform, the bottle mouth is close to the flame, and quickly pour the culture medium into the sterile disposable plate (about 20ml culture medium on the 7cm plate).

c. When set, put the tablet into a plastic bag and put it upside down in the refrigerator (Strep and Tet resistant tablets are light-sensitive and can be stored after being wrapped in aluminum foil).

d. Before using the tablet, open the cover of the tablet, open the UV, blow for about 20 minutes, and dry the surface moisture of the tablet.

2.2.2.3 waste bacterial liquid and plate treatment

It is forbidden to dump or throw away the waste bacteria liquid and plate directly, and all of them should be thrown away after unified sterilization.

2.2 molecular biology

2.2.1 use of enzyme

Those who are not familiar with the operation of molecular experiments must use reagents under the guidance of senior students.

When using the enzyme for the first time, it is necessary to carefully read the instructions, understand the characteristics of various enzymes in the laboratory, and choose the appropriate enzyme and corresponding conditions according to the properties of each enzyme. DNA polymerase: select the PCR conditions recommended by the senior students (especially the extended duration),

rather than just the product description.

The enzyme should be stored in the refrigerator at -20℃, and the enzyme should be removed from the refrigerator when it needs to be added (it is generally added in the last step), put into the low-temperature transfer box (stored at -20℃ in advance) and transferred to the experimental table. If the enzyme is placed on ice, special care must be taken to cover the tube to prevent water!

The gun head needed in molecular experiment must be sterilized and dried to avoid enzyme contamination and icing.

Pay attention to the replacement of the gun head when taking the enzyme solution, and cover it immediately after use to avoid contamination of the enzyme solution. Since the enzyme is generally stored in 50% glycerol and relatively sticky, it should be absorbed from the surface rather than inserted into the bottom of the gun, so as to avoid excessive attachment of the enzyme on the gun head and affect the reaction.

2.2.2 various kits

Plasmid extraction, gel recovery, PCR recovery and other public kits should pay special attention to prevent contamination. Generally, it is enough to follow the instructions, but some key steps, such as the removal of eluent, should be thorough, so as not to affect the yield.

2.2.3 nucleic acid electrophoresis

Each time a new TAE buffer is used, pay attention to the direction of the positive and negative electrodes when placing the tank, and swim from the negative electrode to the positive electrode.

When adding samples, there should be no air bubbles, and the gun head should not break the hole wall. The gun head should be changed for each sample to avoid cross contamination of samples.

Generally, the voltage of electrophoresis is around 120V. When the indicator is located at 2/3 of the glue, it should be avoided to run over the sample and fail to observe the result. After the electrophoresis is finished, clean the electrophoresis tank, glue tank and comb, and then dry.

When observing the electrophoretic results by UV, protective measures should be taken (wearing experimental clothes, gloves, UV goggles, etc.) to avoid being injured by UV.

2.3 biochemical operation

a. Principally, the common reagent should be put back to the original place after use. If you have your own special reagent, you should also keep it in a fixed position.

When storing the reagent, it is necessary to check the physicochemical properties of the reagent (such as whether it needs to be dried at low temperature, avoid light and dry).

Organic reagent should be handled in fume hood. Organic reagents shall be stored uniformly in the cabinet under the fume hood and placed in categories.

b. Organic solvents and solutions containing a large amount of organic solvents should not be put into the refrigerator to avoid the explosion caused by the electric spark caused by the opening and closing of the refrigerator. Regular refrigerator (absolutely forbidden to store flammable solvents, can only to special explosion-proof refrigerator)

c. If any reagent is found to be contaminated during operation, or its physical properties, such as

color and solid liquid, are not consistent with MSDS, it shall be immediately put forward.

d. When handling toxic or dangerous drugs, protective measures should be taken (masks, goggles, double gloves, etc.). The operation should be carried out in the fume hood, and the site should be cleaned immediately after the operation is completed. (if you are first exposed to a dangerous drug, consult your senior first)

e. No albumin staining solution (Fairbanks A, B, C, D) shall be taken out of the fume hood, and the dyeing waste solution shall be poured into the designated sink (located in the fume hood).

2.4 classification and waste liquid management

a. Scrap SDS-PAGE glue

After the decolorization of sds-page adhesive is completed and scanned and backed up, please put the discarded film in the sample box in the ventilation kitchen, do not take up the decolorization box, and the waste shall be poured into the trash bin in the toilet on the 11th floor by special personnel every week.

b. Waste agarose gel

GelRed was used for staining in our laboratory. The excitation and generation lines of GelRed are basically the same as EB, but GelRed is a product with large molecular weight, which is one of the reasons why it cannot penetrate the membrane (safer). Even if it is safer, gloves must be worn when handling the gel, and direct contact is forbidden. Please put the waste glue in the waste box of agarose electrophoresis glue. If the waste glue is full, please pour it into the garbage can in time.

c. Discard syringe needles

Syringe needles are not allowed to be directly thrown into garbage cans, and must be first covered and placed in a discarded needle box (the discarded needle box is made by discarded LB broth kit, and must be marked with marker pen). After filling, the needle box is covered and handled by a designated person.

d. Broken glass treatment

Experimental process, if the triangle bottles, tubes, such as accidentally broke the glass instrument, will first collect broken glass paper parcel together with marker pen mark for glass crumbs, finally puts wrap bag broken glass, glass bag end with organic waste liquid and waste organic reagent bottle by related personnel sent to the waste liquid collecting stations (waste liquid collecting stations appointment: 64251050).

In addition, if there is any glass instrument accident during the experiment, please inform the person in charge of the glass instrument in time for timely purchase.

e. Waste liquid treatment

It is forbidden to directly pour organic waste liquid into the tank. If other students find this situation, please stop it in time.

3. Safety precautions for using common laboratory instruments

3.1 small high-speed centrifuge

It shall be balanced during use, and the sealing cover shall be well covered during centrifugation; If the sample leaks into the machine, please clean it in time (sigma small centrifuge

has been corroded by the leaking organic solvent, please take warning from the students!). ; If there is any abnormal situation do not use, timely report.

3.2 refrigerated centrifuge

It shall be balanced when in use, and the sealing cover shall be well covered in the centrifuge process; Do not walk away before reaching the set speed, so as to find out the problem and deal with it in time. After use, the centrifuge cover should be covered to maintain the temperature before the next use; The last student should close the centrifuge after use, and open the cover of the centrifuge. If the sample leaks into the machine, please clean it in time. If there is any abnormal situation do not use, timely report.

3.3 ultraviolet transmittance instrument

After using the transmittance instrument, the power shall be turned off and the machine shall be wiped clean without any glue residue.

3.4 constant temperature incubator

The fixed temperature of the two incubators in the laboratory has been set, please do not change it by yourself. If you have any special needs, please inform us.

3.5 autoclave: see 2.1.1.

3.6 shaking incubator

In the process of use, if find the liquid overflow, please clean it in time. Overnight training should be booked advanced.

3.7 refrigerator and chromatography cabinet

Please clean up the waste samples respectively in time; The coated tablet shall not be stored for more than 30 days.

3.8 water bath pot

Please replace the water every two weeks (use high pure water to avoid ion scale), and turn off the power after use.

3.9 drying box

One drying box only dries sterile items and the other drying non-sterilized items.

3.10 days flat

Don't move the precision balance at will; Adjust to zero before weighing. Clean up the residual drugs after weighing and keep the balance clean.

3.11 AKTA protein purification system: pressure protection must be set up!

3.12 high purity water system

The person in charge shall change the filter element every month.

Emergency measures

In the event of danger, anyone has the responsibility to help the person concerned control the danger and take first aid measures.

Burns and scalds

Soak burns and scalds in cool water for 10 minutes or until the pain subsides. Remove the ring and other restraints before swelling, and cover with a disinfectant towel. Do not apply sticky tape to the burned area.

Concentrated acid and base

If accidentally splashed on the body, thoroughly rinse the surface with water until there are no residual compounds on the skin. Soap is good for removing compounds. If accidentally a few splash in the test table and the ground, must be promptly wiped clean with a wet cloth.

Replace contaminated clothing and be careful to re-contamination during the process.

Cuts and scrapes

All wounds, big or small, must be treated promptly. Clean the skin near the cut and wrap it up with a sterile bandage. Glass splinters should be carefully cleaned to remove the splinters before dressing. If a large piece of glass is stuck in, do not remove it to avoid serious bleeding and hurry to the hospital.

Electric shock

Turn off the power. If not, use a dry stick to keep the wires away from the victim. If the victim has stopped breathing, give artificial respiration until an ambulance arrives.

Eyes

Eye protection should be done with great care in any job. In the event of an accident, you should: firstly, gently rinse thoroughly with running water. And then get to the infirmary or hospital as soon as possible. At the same time of sending the person to the hospital, the nature of the chemical substance and the treatment process of rescue should be provided.

Hydrogen sulfide

Without an antidote, a few parts per million can kill a person. Move the victim to fresh air and go to the hospital.

Accidental ingestion of chemicals

Do not suck any straw with your mouth. Once it happens, you should rinse your mouth with water, don't swallow.

If swallowed, dilute stomach compounds with plenty of water or milk. Do not induce vomiting. Go to the hospital. When in hospitals, pay attention to the nature of chemical substances and ambulance treatment process.

Phenol (carbolic acid)

Wear gloves and eye patches when using. Quantities greater than 5ml should be handled in fume hoods.

Fire

If you find a small fire, you should put it out in the right way. Always keep yourself between the fire and the door.

Electrical

All electrical instruments need to be regularly checked for insulation and grounding. Report any electrical equipment abnormality immediately. Constant temperature heating apparatus is most prone to fire. You should always observe the temperature change when you use this kind of instrument. Leave the heating instrument regardless of the length of time, to check whether the temperature is constant; Avoid overnight work. Instrument or circuit installed fuse should be correct, when replacing the fuse must be matched with the circuit.

Work alone

If you work late at night or alone, chances are you won't get immediate help to increase the risk. Therefore, try to avoid working out of hours unless your work has to be done. If you are working alone, be aware: 1) if there is no particular danger at work, tell your friends and relatives where you are going and when you will be back. If you can't go back, inform the other party. 2) if there is potential danger in your work, be sure to have other people near your work and inform them of your presence; Check the lab carefully before you leave.

Note: in principle, it is not allowed to work alone overnight in the laboratory.

Security responsibilities for graduate students

As graduate students have the necessary training and considerable experience during their time at university, they are as responsible for their own safety as staff.

Supervisors and laboratory managers shall have the right to take necessary measures immediately if they deem that the graduate students are engaged in unsafe work.