LABORATORY BASICS (appendix to lab_guidelines_OFIZ_2021_v1.pdf)

First Aid:

- First Aid Kit can be found on the wall, 1st floor, behind the glass door to the laboratories; or in the basement, lab no. 1S25 (“Žákovská lab”)
- No food or beverages are allowed in laboratories
- In case of an emergency or a hazardous situation (fire, large leak or flood, dangerous chemical contamination), call **2929** on a landline telephone in respirium

Trash:

- There are trash bins for biological material, plastic, glass, pipets, aluminum and paper
- Laboratory waste belongs to the bins with thick red plastic bag (marked with “Hazard labels")
- Any used/dirty glass beakers has to be washed by water and then put in to the basket in the hall (outside the Tissue culture room), aluminum as well (as we reuse it) - lab technicians will take care of it
- 15ml and 50ml falcon tubes has to be washed as well. After the washing let dry out on racks above sink, (except falcons which were used for patients samples - it has to be bleached and discard in trash (red plastic bag)
- If you work with agar, or agarose solutions or gels, let it hardened first and then trash it in a bin/canister specific for gel waste - **never spill a gel into a sink**
- Every student is responsible for keeping his workspace clean and tidy (put dirty glass and tin foil in the basket in the hall, trash any other used material (paper tissue), spilled liquids, used falcons, empty tips boxes, plates, gloves, etc…)
- **Person who leaves the lab as the last person of the day, takes a quick look whether all machines are turned off (centrifuge, vortex, magnetic stirrer, scales, thermoblock, light in the Cold room; Cell culture room - check the flow boxes, microscopes, water bath)**

Chemicals:

- “Dry” powdered chemicals are located in the cabinet under the Ice Maker in the Western Blot room (“WB room”)
- “Liquid” chemicals can be found in the cabinet under the extractor hood in the WB room ("dirty part” of the room)
- Chemical repository - basement, access only with a permission / Lab Technicians
- Equipment for manipulation with chemicals - shelf under the Ice Maker in WB room
- When you are done with measuring chemicals, make sure you washed all used utensils with dH_2O and let it dry on the rack, also clean work space around scale

Cleaning room:

- Cleaning room is open for any student when something is missing, but it is necessary to be familiar with organisation of the room, and where to find desired supply (clean x sterile; PBS buffer, sterile dH_2O, mQ H_2O, Gelatine,…)

- **The handling of the Dishwasher, Autoclave, and Dryers is only in trained personal's hands!!**

Gloves:

- New boxes with new gloves can be found in the glass cabinet in the hall (outside the WB room)
- New boxes with special robust gloves (intended for work with highly toxic chemicals) can be found in WB room (dirty part, the cabinet on the left side, bottom part) - Only this gloves should be used in dirty part of WB room!

Ethanol stock sources:

- 70% Ethanol: available in handy sprays around the labs, for disinfection
- 70% Ethanol 5l stock canister: on the sink in Bench room/Cell tissue room (back part)
- Denatured Fuel Ethanol (96% Ethanol + 1% Gasoline) poured into dH_2O is used for mixing 70% EtOH in flasks
- Ethanol Absolute A.G. (Stored in dark brawn 1000ml bottles, can be found around Bench room/Cell tissue room) is used for experiments, buffers, samples…)

Filling tips:

- Bench room – empty boxes are collected in Bench room – next to the sink. Students who have "filling tips duty" each week are responsible for filling the boxes with new tips. Spare bags with new tips can be found in the cabinet under the sink or in the glass cabinet in the hall. Lab technicians will collect full boxes and will take care of the sterilisation
- WB room – Tip boxes from WB room don't need to be sterile - they are filled in "dirty" part of WB room; never ever take any empty boxes (or anything else) from WB room!!

Most commonly used buffers / preparatory duties in the lab:

Preparation of gel electrophoresis:

Prior of making an agarose gel, it is handy to start with preparing the gel tray (secure it with duct tape) and having the appropriate combs that fit the tray.

• "Small gel" **(70ml):**

-70ml TAE

- Agarose (amount is based on desired concentration) for DNA gel electrophoresis (cabinet in the WB room under Icemaker) – carefully dissolve in the "dirty" microwave until the solution cleared up (takes approx. 8min at medium voltage)

- **Work in the extractor hood!!** add approx. 4µl Ethidium bromide (EtBr; fridge 112/6) to the warm gel, and the pour it to the gel tray
- Leave it for about 20 min. to harden

• "Large gel" **(150ml):**

- 150ml TAE

- Agarose (amount is based on desired concentration) for DNA gel electrophoresis (cabinet in the WB room under Icemaker) – carefully dissolve in the "dirty" microwave until the solution cleared up (takes approx. 8min at medium voltage)

- **Work in the extractor hood!!** add approx. 8µl Ethidium bromide (EtBr; fridge 112/6) to the warm gel, and the pour it to the gel tray
- Before you ran the electrophoresis, make sure that there is enough of the TAE buffer in the buffer tank

When you are done, make sure to carefully clean your workplace, used supply, and **trash the used gels in the special canister (yellow bucket with red lid).**

Preparation of 1x TAE buffer (1l):

- 20ml of 50x concentrated TAE buffer
- Fill the bottle up with dH_2O to 1l
- Can be only prepared in dirty part of WB room

Preparation of 10x running/transfer buffer (1l):

- 144g of Glycine
- 30,3g of Tris powder
- If you want to pour larger volume of different chemicals into the bottle, use the glass funnel (should be next to the sink, or in the cupboard under the scales)
- Fill up the bottle with dH_2O to 1l
- Place the stirrer magnet to the glass and leave it properly dissolve on the digital magnetic stirrer machine (next to the scale)

Preparation of running buffer (canister 10l):

- 1l 10x Running buffer
- 50ml 20% SDS
- Fill the bottle up with dH_2O to 1l

Preparation of wash buffer (10l):

- 50ml of 2M Tris pH= 7,6
- 200ml 5M NaCl
- 80ml of 10% Tween
- fill up the canister with dH_2O to 10l

Preparation of 20% SDS (500ml):

- 100g SDS
- If you want to pour larger volume of different chemicals into the bottle, use **the glass funnel** (should be next to the sink, or in the cupboard under the scales)
- Fill the bottle up with dH_2O
- Place the stirrer magnet to the glass and leave it properly dissolve on the digital magnetic stirrer machine (next to the scale) also keep the temperature during stirring at 60 ^oC. If you are leaving the lab, don´t forget to turn off the heating.

Water bath solution:

- 5ml AquaClean solution (little bottle in the shelf under centrifuge in Cell tissue room; photosensitive solution - has to be store in dark!)
- Fill the bottle up with dH_2O to 1l

Preparation of LB (500ml):

- Add 12,5g LB powder (cabinet in the WB room under Icemaker)
- Fill up with dH_2O
- Solution needs to go through sterilisation
- Mark the bottle properly, with the date of preparation

Preparation of LB agar (1000ml):

- Add 25g of LB powder and 12g of Agar (Agar Agar; Penta).
- Fill the bottle up with dH_2O
- Mark the bottle properly, with the date of preparation
- Solution needs to go through sterilisation

Preparation of ATB aliquots: (ATB aliquots can be prepared “semi sterile” in bench room)
ATBs (aliquots ready to use) are stored at -20 °C (freezer in bench room 113/5, middle shelf). Stock powder can be found in the fridge 113/2 at +4 °C

- **Ampicillin** (aliquots **25mg/ml**) - (e.g. 1250 mg of Amp stock powder dissolve in 50% ethanol solution in 50ml falcon tubes and leave them at -20 °C (113/5))
- **Kanamycin** (aliquots **25mg/ml**) - measure the amount on the analytical scale with using 15ml falcon tubes, fill with dH_2O based on measured weight; (e.g. for preparing of 15ml you need 375mg of powdered Kan). Prepare single aliquots of 1,5ml tubes Kan and leave them at -20 °C (113/5)

ATB plates preparation:

- Bacteriological plates can be prepared “semi sterile” in bench room, or sterile in the flow box (Lab technicians)
- LB agar can be found downstairs in the Cold room
- Prior of preparing plates, heat up the LB agar in a microwave (“clean microwave”, Hyršl lab 115)
- Bacteriological plates can be found in the glass cabinet outside of the WB room in a hall
- Add ATB to the warm LB agar (temperature should not be to high (ATB would be degraded), when you can comfortably hold the glass in your hand
- Quickly mix the ATB in the LB agar, and pour on the bacteriological plates
- Leave it cool down and harden on open plates for at least 10-15min
- Once the agar harden, cover the plate with the lid, and secure with Parafilm
- ATB plates are kept in the Cold room, bottom up, clearly marked with a day of preparation
- **Ampicillin**: **4ml of ATB / 1l LB agar** (With expiration time **4 weeks)**
- **Kanamycin**: **2ml of ATB / 1l LB agar** (With expiration time **6 weeks)**

Preparation of 5M NaCl (500ml):

- 2,5 x 58,44g powdered NaCl
- If you want to pour larger volume of different chemicals into the bottle, use the glass funnel (should be next to the sink, or in the cupboard under the scales)
- Fill the bottle up with dH_2O
- Place the stirrer magnet to the glass and leave it to properly dissolve on the digital magnetic stirrer (next to the scale)

Preparation of PBS (2l; Lab technicians):

Add:

- 16,4g of NaCl
- 5,8g of $Na_2HPO_4*12H_2O$
- 0,4g of KH_2PO_4
- 0,4g of KCl
- Fill up with mQ H_2O to 2l
- Place the stirrer magnet to the glass and leave it properly dissolve on the digital magnetic stirrer machine (next to the scale)
- Then, the solution needs to be filtrated thru filter device in the Bench room
- Needs to go through sterilisation

Preparation of TRIS buffer (incl. pH correction; Lab Technicians):

- TRIS (powder; cabinet in the WB room under Icemaker)
- Specific amount of a TRIS powder is written on the Ice Maker, it depends on a volume of solution and desired final concentration
- Fill up with mQ H_2O to 3/4 of the final volume
- Place the stirrer magnet to the glass and leave it properly dissolve on the digital magnetic stirrer machine (next to the scale)
- When the solution is dissolved, check the pH and adjust it by using HCl

- When the pH is OK, fill up the bottle with dH_2O
- Leave it to mix overnight on the magnetic stirrer
- Next day - check the pH, adjust it accordingly (when the pH got too low, you can use NaOH to adjust)

Pipette cleaning and calibration:

- Contaminated filters can be replaced only on a 5ml pipette
- www.youtube.com/watch?v=q0o-VBMVKio (How to disassemble, assemble, clean and grease the Eppendorf Research® plus mechanical pipette)
- We are capable to calibrate only 200µl, 1000µl and 5000µl pipettes (Lab Technicians) using the analytical scales in the WB room
- Other pipettes have to be fixed by the manufacturer (Eppendorf at Eppendorf provider, Labnet pipettes at KRD provider)

Ordering system:

It is desired that every student should check on any material needed for his work. When you see that something is running low/missing, you are obligated to let you supervisor, Lab Technician or Lab manager (Ingrid Prišticová) know about this situation, so we can order new ones.