

## Lab manual

As you noticed, **it's nobody's work to clean after you...** So please always keep your and the common working place in order. It's also important for your good health and everybody's safety. Remember when there were bacteria spilled in the centrifuge until it started to stink like hell? But it took us quite a few days before we noticed... not very sterile indeed. Or do you remember when somebody spilled ethanol all over the burner and Ivka's hand caught fire? For cases like these, please try not to spill chemicals all over the tables (methanol, acrylamide...), mind your bacterial work and be careful in general.

### Waste

There are two types of waste bins in the lab, which are discarded in specific ways – the communal waste and biological waste. Biological waste bins are the big ones – **NEVER** put the liquids in them – the bags are not water-proof. The pipette tips have to be put first in the plastic flasks so they do not punctuate the bag. In these bags put only things contaminated with biologic samples – their disposal is expensive. All the wrapping material put in the plastic recycling box or communal waste.

The communal waste bins are the small ones – never put gloves or any lab plastic in it. Do not throw the paper in it – we have a special box on the corridor for collection of paper for recycling!!! The broken glass or empty glass bottles put in the buckets next to the paper box – white and stained glass separately. Empty paper or polystyrene boxes we keep in the mezzanine. We collect the empty PET bottles for the dirty tips – they are either on the glass cupboards in the corridor or in particular labs.

The waste is collected in the small yard which is on the way to the botanic garden. We have two special containers for the biologic material (the big bags from the big bins). There is also the special container for paper and glass. Near the entrance to the dean's office is the container for PET bottles. All other plastic material will Vendulka drive to Jihlava, where it is recycled. The box for plastic is facing the paper box on the corridor and the one for the wrapping material is also in the cultivation room under the centrifuge.

### Safety in labs

The priority is **YOUR** safety, the safety of biologic samples follows. On these premises wear the gloves in the labs. In case of any injury always inform your supervisor. There is the first aid box in the corridor. When using any evaporating or volatile chemical, use the fume hood. The liquid nitrogen can cause the freezing marks, so work with it carefully with the thick gloves.

### The western blot room

The most dangerous is the western blot room. You should presume that everything in this lab is contaminated with acrylamide, so do not touch anything without the blue gloves. Do not wear these blue gloves outside the western blot room!!! The beakers, racks, boxes, etc. used in this room is labeled “WB” and these things must not leave this room. On the other hand, do not bring any non-labeled things inside. Besides acrylamide there is also risk of toxic exposure to Ethidium Bromide and Methanol in this room. Use fume hood as much as you can.

Used gels put in the container with blue cover (the reuse of empty containers of NaCl or glycine) – so we can minimize the risk of dried acrylamide particles flying around in the air we breathe.

We have four common 2l bottles of transfer buffer. Each time you run it check the current – one transfer unit should not have current higher than 500 mA! When the current is higher discard the

transfer buffer to the sink and make a new one. The bottles are numbered so, please, avoid mixing of their content!

When technicians do not manage to tidy away the dry dishes in the morning, please do that on your own so you do not mix up the dry and wet dishes.

When pouring the running or transfer buffer, try to keep the table paper dry (for example by putting the tray under the container).

When you use the scales, do not move them and after work it must be clean!!!

### **The cultivation room**

The work in this room is described in “Cell and tissue culture work” document attached.

### **Stock chemicals, films, buffers**

The most annoying complication is lacking chemical when you are in the middle of the experiment. To avoid this, please, be very watchful when using any chemical and always when something begins to run out, inform the appropriate person (see above) to order the new chemical. The most often runs out the protein standards, chemicals for qRP-PCR, ECL, antibodies. In case you use any of common buffers, mix a new one. If you don't know how to do it, ask technician or some senior student. Thoughtfulness helps us all to work the most efficiently!!!

### **Pipettes**

All the pipette sets should be labeled with the owner's name. The owner is also responsible for their preciseness and cleanness. Once each two months the owner has to check if the pipettes are OK and in case of need calibrate it. Each time you suck any liquid in the pipette, it should be cleaned (dismantle if possible; wash with ethanol, let dry).

### **Pipette tips**

When you have some spare time during the experiments, please, fill the tip boxes. It is for a common good. Use gloves when doing it. PCR tips are done with the tweezers. Leave the filled boxes for sterilization.

### **pH meter**

The measuring electrode is kept in the distilled water and the small hole in the upper part has to be covered by the blue cover gum. Before measurement remove the cover gum and turn on the pH meter. It goes to the standby mode pretty quickly, so when the pH sign on the right side stops blink you need to push the “measurement” button. After measurement or between the different liquids clean the electrode with distilled water. The pH meter is turned off with pushing the same button as when turned on (only longer).

### **The duty of the last (wo)man leaving**

Close all the windows (the exclusion is the room with -80° freezer)

Turn off the non-working machines

Turn off the lights

Close (not lock) the doors on the corridor (přepážky)

There are the spare keys in the box in the main bench room for case of need.

