SOP for Mayer Lab Cell Culture

Written by Daniel Estes, 2007

Cell Culture Hood

The Cell Culture Hood is made for applications that need to be "sterile" (free of bacteria and particulate). As the name implies, all cell culture work should occur inside the hood, as contamination with bacteria can destroy cell lines, affect growth mediums, and generally ruin any experiment that involves incubation. **As such, it is EXTREMELY important to keep the inside of the cell culture hood VERY clean:**

1. Before opening the glass pane, turn on the blower (switch on left). **Never open the hood without having the blower on!**
2. Open glass pane at a level below the marked "maximum" on the left side of the hood.
3. Let blower operate for 15 minutes prior to use in order to establish a "sterile" environment inside the hood. During this 15 minutes, it is a very good idea to wipe the metal surface of the hood with 70% ethanol. Be sure to use 70% ethanol, as 100% ethanol will evaporate too quickly to effectively kill microbes in the hood! 10% bleach can also be used, although it is harder to wash out of the hood.
4. Whatever goes into the hood (pipettors, tips, waste jars) should be sterile. Wipe down items with 70% ethanol before putting into hood.
5. If there is a spill of some kind in the hood, clean it up (with Kimwipes and 70% ethanol) immediately, as the inside of the hood can stain VERY easily.
6. **Dispose waste in Biohazard containers!** Especially cell waste and blood – must go into the Biohazard waste container! Put supernatants and plasma (of blood) into plastic waste containers (25 mL Falcon tubes) so they can easily be put into the Biohazard waste.
6. NOTE: the cell culture hood blows air out, meaning that airborne and dangerous fumes are blown out of the hood towards the user. **Do not open toxic substances (such as 100% mercaptoethanol) or work with dangerous fumes inside the cell culture hood!**
7. **Wear gloves when operating inside the hood.** Do not pass bare skin over/around your sample, as the sample can become contaminated.
8. When you are done using the hood, **you must wipe the inside of the hood with 70% ethanol** before leaving and closing the glass pane. Wipe down the ENTIRE metal surface of the inside of the hood thoroughly with 70% ethanol.
9. After wiping down the hood at the end of a session, close the glass pane COMPLETELY and then turn off the blower and any light. Also, do not leave the cell culture hood on for extended periods of time, as the HEPA filter that ensures a sterile environment does not last forever.

Large Centrifuge (Thermo Electron)

The large centrifuge has three adaptor modules that can hold 15 mL tubes, 10 mL tubes, and 5 mL Falcon tubes (for flow cytometry). The centrifuge is made to operate at speeds of 100 – 2000g. **The most important aspect of this centrifuge (as well as any centrifuge) is to balance the rotor of the centrifuge (i.e. the weights on opposite sides of the centrifuge need to be symmetrically distributed):**

1. Place your sample into the centrifuge containers (including screw-caps). **You must use BOTH containers, and each container must be the same weight.**
2. **Balance the containers,** which should contain your samples and also one "blank" tube for balancing purposes:
    - Put one of the containers (including the cap – the caps are not all the same weight!) onto the digital scale.
    - Zero the scale.
    - Place the other container onto the scale and then add/subtract water from the "blank" tube until the scale reads less than 0.1g.
    - ***You MUST balance the containers (including samples and screw-caps) to within 0.1g or the centrifuge can break!***
3. Before opening the centrifuge top, set your speed (far left-side – keep speed in g's), time (to the right of the speed), and temperature (middle panel – temperature takes 5 minutes to cool, but 30 minutes to heat back up). **Do not EVER change the rotor number (should ALWAYS be 243).**
4. Open the centrifuge top with the button below the red stop sign. The lid is fairly heavy, so pull up on the top opening after hearing a "click".
5. Close completely the top, and then press the green arrow.
6. When not in use, keep the centrifuge closed to not allow dust and other matter into the chamber of the centrifuge.

Incubator (Thermo)

The incubator is always set to 37 degrees at 5% CO_2. While the incubator has O_2 control, it is not currently hooked up and is naturally ~16%. Do not change these settings! **Like the cell culture the hood, the incubator needs to be sterile – you must be careful about what is put into the incubator (and also how you reach into the incubator).**

1. **It is CRITICALLY important to keep the incubator door closed as much as possible.** Temperature and CO2% are vitally important parameters to cells, and these values can

rapidly change if the door to the incubator is left open for too long. Know where your sample is before opening the incubator!

2. Wear gloves whenever dealing with the incubator.
3. Open the outer door of the incubator.
4. Open the glass door by rotating the black knob on the right by one-quarter turn counter-clockwise.
5. Quickly retrieve/place your sample. If you are placing a sample into the incubator, make sure it is clean by wiping the bottom of the sample with 70% ethanol.
6. Immediately close the glass door by rotating the black know to the left once the door is shut.

Regular maintenance:

1. Change the **water pan** at the bottom of the incubator. The humidity of the incubator is high to prevent evaporation, and the water pan maintains this humidity. However, this water is a prime source of bacteria. Therefore, it must be changed regularly (once a month).
2. Cleaning of the **inside of the incubator** by wiping all surfaces with 70% ethanol (once every 2 months).
3. Change **HEPA filter** (once every 6 months).

Small Centrifuge (Eppendorf)

The small centrifuge (Eppendorf model) is made to operate at high g's (20000g max) with either the 2, 1.5 or 0.75 mL Eppendorf tubes.

1. Set the speed of the centrifuge using the middle panel. Speeds can either be in g's (RCF) or RPM – you can switch modes by holding down both the UP and DOWN arrows at the same time.
2. Set the time and temperature of the spin.
3. Open the top cover by pressing the OPEN button.
4. Open the top black cover by rotating the top knob to the left.
5. Place your sample in the appropriate slots – NOTE: **the weight MUST be distributed symmetrically on the rotor** by placing samples on exact opposite sides of the rotor. If you have only one sample, use a blank Eppendorf tube of the appropriate size on the opposite side from your sample.
6. Put the black cover on the inside chamber by rotating the top knob to the right, close the top, and press START

Water Bath

The water bath is set for 37 degrees Celsius and should not, in general, be changed from this value. If desired, the temperature can be changed by the panel on the left of the water bath.

1. Turn on the water bath. Note: the "over" temperature is set at 10, and this value is necessary for the bath to be able to heat up to 37 degrees C. Also note that the bath takes about 10 minutes to heat from 20 degrees C to 37 degrees.
2. Place your sample in the bath and let heat to 37 degrees C (pretty easy). However, some samples you will want to heat gently, especially from the frozen form to 37 degrees. For FBS, for example, it is a good idea to first thaw the FBS in 4 degrees overnight rather than heat directly to 37 degrees from the frozen state.

Maintenance:

- The water in the water bath must be changed at least once a month.
- It is not easy to move, so the recommended way to change water is to scoop water out using beakers.
- Excess water can be removed using Kleenex to soak up the water.
- **You must used DISTILLED WATER** (not deionized and not tap water) that you should purchase from Kroger, e.g.
- Typically, fill the water bath with ~0.5 gallons (2 L) of distilled water.