# Protocol for using the Microfermentation robot

This protocol is intended as a guide on how to set up and use the microfermentation platform.

## 1 Materials

- Microfermentation platform
- Bunsen burner and matches
- 70% Ethanol for sterilization
- Scissors
- 4 Macro cuvettes (4 ml, polystyrol)
- 4 sterile needles
- 4 sterile filters (0.2 µm)
- Parafilm or adhesive tape (ca. 1 cm width)
- ~ 6 ml liquid medium (2 ml per cuvette)
- Starter cultures

## 2 Preparing cuvettes

1. Carry this out next to a flame
2. Wash the cuvettes with 70%vol Ethanol.
3. Rinse and let them dry next to the flame

## 3 Gathering your stuff

*While the cuvettes are drying*

1. Take the media and starter cultures out of the fridge and put them on your workbench.
2. Cut 1cm x 1cm rectangle squares of parafilm. Alternatively use short strips of adhesive tape.

## 4 Setting up the robot

1. Turn on the robot and edit the settings as desired. *The most important settings are the duration of the experiment and the intervall for OD measurements.*
2. Record the output file name in your lab book.
3. Calibrate pump:
    a. Start the pump test routine.
    b. Fill another cuvette with water.
    c. Connect a filter and needle to one of the air outlets.
    d. Submerse it in the water and adjust the valve to a desired level of aeration. *This depends on the organism you use and the expected foaming. Preliminary tests may be required for the optimal level. In general, bubbling should be strong enough to mix the volume in the cuvette but not drive all the liquid out of the cuvette during fermentation. It might be feasible to use an anti foaming agent.*

*Return to your sterile workplace.*

## 5 Making blanks

1. Fill each of the cuvettes with 2 to 2.5 ml of medium. *The actual amount depends on how you inoculate. In general, the total volume during fermentation should not exceed 2.5 ml*
2. Seal the cuvettes with the parafilm squares.
3. Load them in the fermenter and close with the black top.
4. Run the Calibration routine to optimize the light output of the LED and get a blank value for your media for subsequent OD-measurements.

## 6 Inoculation

*This part of the protocol varies on the organism and starter culture you would use. In general:*

1. Add a small quantity of liquid starter culture or pick a colony from a plate and put them into the cuvettes.
2. Seal the cuvettes again and put them back into the measuring cells.

## 7 Starting the fermentation

1. Take the needles and filters out of the sterile packaging.
2. Screw the needles in the filters and loosen the needle caps slightly.
3. Mount them on the air outlets. Finally remove the caps and put the Aeriation unit on top of the measuring cell.
4. Make sure to push it all the way down.
5. Start the fermentation by clicking on the menu point

## 8 Getting your data

1. Remove the µSD-card and plug it into your computer.
2. Copy the file with the name you recorded and import it into your favourite calculation software.
3. Put the memory card back into the robot.