Quick operation guide to EmulsiFlex-C3 high pressure homogenizer

0 – Notes prior to use

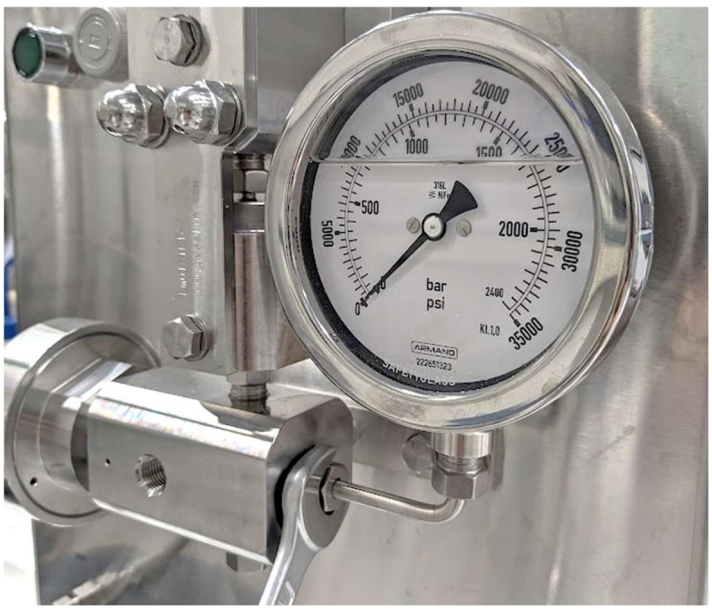
The recommended sample volume is 25-250 mL. The addition of DNAse I is highly advised, since the homogenizer does not shear DNA and the sample will become very viscous.

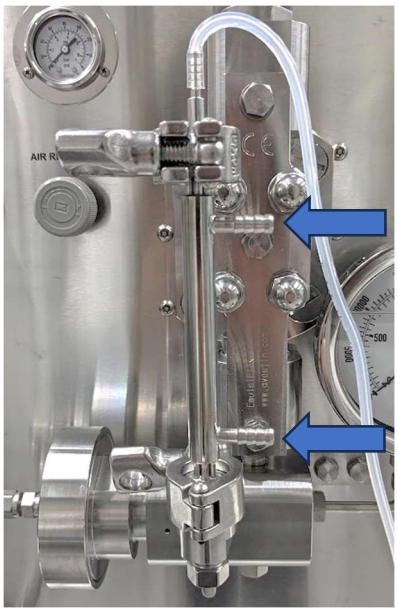
1 – Assembly

The sample holder is in the fridge next to the French press, bottom drawer. Please make sure to collect the small O-ring as well. Place the O-ring on the top fitting, as shown below:

Place the sample holder on top and hold it in place with the metal clamp. This only needs to be finger tight.

Place the pressure gauge on the side, as shown below, tightening it with a 17 mm wrench. No need to overtighten. Place the extruder in front, as shown below, tightening it with a 17 mm wrench. No need to overtighten.





OPTIONAL: Connect the cooling tubes from the water cooler to the extruder (blue arrows).

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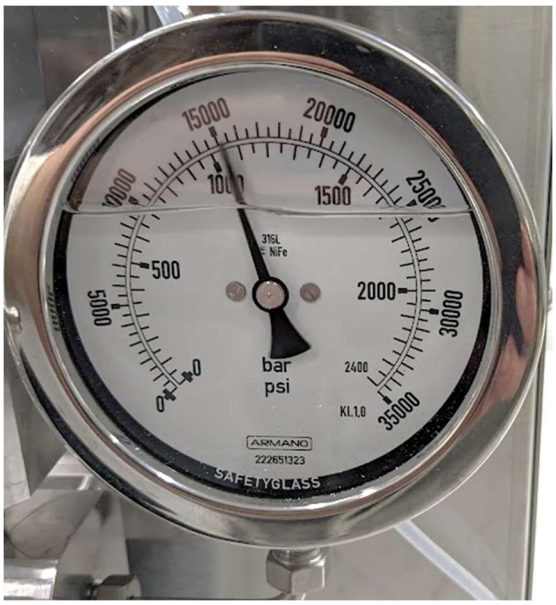
Open the air valve on the wall (3 bar should be enough for normal operation). Ensure the air valve on the homogenizer shows a similar value.

2 – Use of the equipment



Turn the main switch on, in the back. The red STOP button should light up.

Add some of your lysis buffer (25-50 mL) to the sample holder Place the extruder tube in a collection container.

Rotate the button as the arrows in the STOP button indicate. It will unlock the button. Press the green button, it should light up.

Move the air regulator clockwise, noticing the increase in the air pressure on the valve above it. The pressure gauge should eventually start moving, pushing your buffer out. This guarantees the system is functional. Stop the pressure by moving the air regulator counterclockwise until the air pressure goes back to 0. **Always leave a small amount of buffer as no air should be pumping into the system when the pressure is on.**

Add your sample to the sample holder and, once again, increase the pressure by moving the air regulator valve clockwise. As the machine disrupts the cells, pay attention to the **large homogenizing pressure gauge**. Disruption of *E. coli* cells requires only 15000-17000 psi, with *Saccharomyces cerevisiae* needing 22000 psi. The pressure the cells are exposed to is regulated by the air regulator.

When the entirety of the sample has gone through, press the red button to stop. You may restart the procedure if another passe is needed. Previous tests revealed two passes should be enough to disrupt *E.coli*; yeast cells require ~5 passes. A single pass of 250 mL takes approximately 10 minutes.

After the passage, add once again a small amount of your buffer (25-50 mL) and pump it through the system **almost** to completion. This will remove any leftovers of your sample, and once again, prevent air from going in.

Note on small volumes:

Because small sample volumes (< 50 mL) are passed through the machine very quickly, it is recommended you hold the line in such aa manner as to create a cycle (see photo below). After a few passages the cells should be disrupted and you can move the air regulator valve counterclockwise until the air pressure turns to 0, leaving as little of your sample as possible. Then you can add more of your lysis buffer to remove the leftovers of your sample.

3 – Cleaning up

Make one pass with 250 mL water and another passage with 20% EtOH to clean the system. **Again, always ensure the total amount of liquid in the holder never goes to 0 (ie, no air is being pumped).** 250 mL of a liquid with no viscosity takes ~10-15 minutes to pass through the system.

Disassemble the holder. Clean the O-ring and fitting with 70% EtOH. The holder itself should be washed, disinfected with 70% EtOH and dried before placing it back in the fridge.

Before loosening the homogenizing pressure gauge and extruder, it is recommended to place a container below, since some liquid may be released. Clean the connection and the fitting with 70% EtOH. Note the extruder can be autoclaved.

Close the air valve at the wall.

4 – Troubleshooting

If the machine just stops, it is likely the air pressure was too high. If the pressure goes above 30000 psi, the system automatically shuts off. Press the red button, release all the air with the air regulator valve and shut down the system in the main switch. Re-start, making sure not to introduce too much air pressure in the system. If the problem persists, contact Turku Protein Core.

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If, due to viscosity, the sample is stuck, there is a simple way to clean the system. Press the red button to shut the machine off and release all air with the air regulator. In addition, drop the air flow to 1 bar in the wall. Connect the extra air valve to the top of the sample holder (see photo below). The air will force the sample to go from the holder to the extruder, cleaning any clogging in the process. Note that the sample thus removed has not been disrupted. Make sure there are no clumps, dilute the sample if needed and try once more.