

Gloves and goggles must be worn when working with pathogenic, toxic, or corrosive materials.

Do not run the machine dry for more than a few cycles, this will significantly reduce the lifetime of the seals in the pump. In an EMERGENCY, press **STOP** button immediately, do not adjust pressure gauge. Adjust pressure gauge after stopping.

Starting Homogenizer:

1. Switch on main power supply located on the rear left of the EmulsiFlex-C3. **Deliver N2 gas at 75 psi.**
2. Turn on chiller using power switch, then pressing power button, then press center arrow button to start flow (on screen, “play” icon will change to “stop” icon). Wait until chiller is at 4°C.
3. Apply nitrogen tubing on reservoir cap, push out EtOH, fill reservoir with DI H2O & flush 3-5 times.
4. Fill reservoir with DI H2O. Twist red **STOP** button clockwise. Press green **START** button to start pumping DI H2O into chiller column at 4°C. This will direct DI H2O internally. Wait until a steady outlet stream is achieved. **IMPORTANT:** always allow system to fill with liquid entirely before adjusting pressure knob
5. **Adjust pressure slowly** by turning air pressure knob clockwise to ~40 PSI, achieve 10 strokes at 15-20K PSI then reduce pressure to 0 PSI. Liquid will not come out if pressure was adjusted too quickly.
6. Finally, press **STOP** button to change to outlet path. Flush remaining DI H2O using nitrogen tube.

Small Sample Volume Lysis/Homogenization:

1. **The minimum sample volume is 10 mL**, samples must be pre-homogenized using a 16-gauge needle or by another homogenizer. This will prevent chunky pieces from clogging the homogenizer.
2. Add sample in the reservoir and direct end of outlet tubing so sample is directed back into reservoir forming a closed loop. Unlock **STOP** button, press **START** to direct internally, when sample comes out evenly, **increase pressure slowly** to ~40 PSI by turning the pressure knob clockwise.
3. Keep **homogenizing pressure between 15K-20K PSI or as needed**. The suggested time for 100 mL sample is 10 min, however time will be based on sample. Homogenize sample as needed.
4. When finished homogenizing, place outlet tubing in collection container before pressing stop to prevent sample loss. Reduce pressure to 0 PSI and press **STOP** to direct homogenate to collection container.

Cleaning Homogenizer After Use:

1. Chiller must at 4°C. Add room-temperature 0.5 M NaOH solution to reservoir, direct to outlet tubing until steady steam is achieved, form closed loop, coat internal with NaOH by pressing **START**, press **STOP** button and allow NaOH to remain in the homogenizer for 30-45 min (not longer, will corrode!). Purge NaOH with N2.
2. Cleaning outlet tubing: add small volume of 10% Contrad 70 detergent, then fill with DI H2O to make diluted detergent solution. Flush out to outlet tubing with diluted detergent solution. Repeat 3 times.
3. Cleaning internal: unlock **STOP** button, press **START** to direct internally, wait until you achieve a steady outlet stream, **adjust pressure slowly** by turning air pressure knob clockwise, achieve 10 strokes at 15-20K PSI then reduce pressure to 0 PSI. Press **STOP** button to direct externally, flush out solution with nitrogen tubing.
4. Fill reservoir with DI H2O, repeat internal cleaning 5 times to remove detergent.
5. Keep EtOH in system: fill reservoir with 70% Ethanol, direct internally at ~40 PSI, achieve 10 strokes at 15-20K PSI then reduce pressure to 0 PSI to have EtOH in homogenizer while not in use.
6. **Turn off: chiller, nitrogen source, and homogenizer.**

