French Press SOP

Steven Backues, 2016

Do not use this equipment unless you have been trained by Dr. Backues. This SOP is for reference purposes only.

*Review the **critical reminders** on the last page every time that you use the instrument to avoid damaging it or injuring yourself.*

Procedure

1. Resuspend the cell pellet in an appropriate volume of ice-cold lysis buffer (typically 1 to 4 ml per gram of cell pellet)
2. Removed the chilled 40K pressure cell and accessories from the shelf in the cold room. Make sure that the piston and the inside of the cell are clean and free from any particles.
3. Lubricate the O-rings on the piston, the base, and the flow valve with 100% glycerol using a cotton-tipped applicator (Q-tip)
4. Insert the piston into the top of the cell with a gentle twisting motion. Insert it to at least the "max fill" line (accommodates 35 ml); deeper if your sample volume is smaller
5. Screw the sample outlet tubing and the flow valve into the base. Do not screw the flow valve all the way in – leave it open.
6. Place the cell, inverted, on the filling stand – see Figure 1.
7. Pour your sample into the cell. The liquid level should be ~2.25 cm from the top of the cell (use the provided measuring stick). If it is lower, air will be introduced into the cell causing it to spurt when processing. If it is higher, some will be lost when the base is put on. The piston can be moved to adjust the level of the liquid until it is at the right level.
8. Place the base, with the flow valve open, into the cell. A little bit of your sample should be forced through the outlet tubing – this ensures that there is no air in the system.
9. Carefully close the flow valve by ***gently*** tightening it until just finger tight. Note that many rows of threads will still be visible. **DO NOT OVERTIGHTEN!**
10. Ensure the French Press platform is all the way down
11. Place the filled cell right side up on the French Press platform and seat it against the three centering pins. *BE SURE that the cell is pushed firmly back against* **ALL THREE PINS**. *If the cell is not centered, the pressure could cause it to shoot across the room (or through you).*
12. Swing the cell clamp into position and tighten the thumb screws to secure the cell into place.
13. Make sure that the handle of the piston is **perpendicular** to the thumb screws so that it doesn't break them as it is pushed down.

Figure 1: 40K cell in the filling position.

14. At this point, your set-up should look exactly like Figure 2. Do not proceed until it does (otherwise you might be about to die).
15. Place some sort of collection tube at the end of the outlet tubing to catch your sample.
16. Move the ratio-selector handle to the "Down" position. Turn the press on (black switch on front of machine). With the selector still on "Down," turn the pressure increase knob to the desired gauge pressure. Refer to the chart on the front of the press to see how the gauge pressure that you are setting will convert to actual pressure inside the cell. For *E. coli*, a cell pressure of ~10,000 PSI should be sufficient, which would correspond to a gauge pressure of ~700 on the "high" setting.
17. Once the pressure is set, and making sure that the flow valve is ***gently*** closed, turn the ratio selector the either "medium" or "high" as desired to start the process.
18. As soon as the top of the piston reaches the press platen, the sample inside the cell will be pressurized. Adjust the flow valve so that the sample flows out of the outlet tube at no more than 15-drops per minute. Higher flow rates will allow the pressure inside the cell to decrease, and therefore cause poor lysis. (The lysis occurs as the cells pass suddenly from the high pressure cell into atmospheric pressure). **NOTE:** The flow valve is very sensitive – a tiny movement can have a big effect. In order to adjust the flow rate, tap the valve with one finger to open or close it the slightest bit. Be careful never to over-tighten the valve – only close it as far as needed to get the desired flow rate.
19. Monitor the flow rate and pressure as the sample is processing, and adjust as necessary. You will need to frequently open the flow valve a little bit more in order to keep the sample flowing. To get good lysis, open the valve very slowly, by tapping it, so that the flow is always drop-wise, never a stream.
20. As soon as the "STOP" line on the piston has reached the top of the cell, stop the process by turning the ratio-selector handle to DOWN. **NOTE:** There will be some sample loss due to material left in the valve ports and drip tube. Do not try to squeeze out the last drop sample. You will damage the piston, closure plug and cell body. **Do not allow the piston to go beyond its "STOP" position.**

Figure 2: Proper set-up of the cell in the press. Note how the handle is perpendicular to the thumb screws on the cell clamp.

21. For more complete lysis, return the sample to the cell for another pass (go to Step 7)
22. Once you are done with the press:
    a. Return the platform to its lowest position
    b. Turn the pressure increase knob all the way down (to the left) to return the pressure to 0
    c. Completely disassemble the cell. Rinse the piston, cell and base inside and out with deionized water (use a squirt bottle to rinse inside the base). Dry all parts and return to their box. Return the box to the drawer in the cold room.

Critical Reminders: review these every time to protect yourself and the instrument

1. Remember to lubricate the O-rings with glycerol and make sure that the piston and cell are clean before using.
2. Do not overtighten the flow valve. Be *very gentle* with this or you will damage the base of the flow cell, which is very expensive to replace. It should only be finger tight.
3. Be sure to set up everything exactly as shown in Figure 2, with the cell positioned against all three pins and the handle perpendicular to the thumb screws. This press can be dangerous if misused.
4. Do not exceed the pressure printed on the cell
5. Do not use the press to push the piston past the “STOP” line.