

# DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

## SAFE OPERATING PROCEDURE

### FRENCH PRESS

#### Purpose:

To provide safe operation and instruction for the French Press to Research/ Teaching staff members in the Department of Molecular and Cellular Biology.

#### SAFETY PRECAUTIONS:



- All operators must receive training prior to using the equipment. **Please arrange training by contacting Jamie Jones (Department Support Technician), ext. 53816/ SCIE 4482.** It remains the responsibility of the supervisor to ensure their personnel are adequately trained. Report any issues with the French Press to Jamie Jones.
- Never exceed the pressure limit on the cell you are using. Exceeding these limits can cause the pressure cells to fail, and potentially cause serious injury.

CELL TYPE	FRENCH PRESS CELL RATING			
	Cell PSI	MAX GAUGE	Max Volume	RATIO
40 K	40,000	2520	35mL	HIGH
Mini Cell	20,000	900	3.7mL	MED
<b>DO NOT EXCEED RATING</b>				

- Keep hands away from the point of operation when the press is in use.
- It is imperative to protect the piston from foreign matter at all times (i.e. dirt, glass, sand etc.)
- Thoroughly clean the French Press and pressure cell /piston/closure plug after every use.
- Ensure Personal Protective Equipment is worn (lab coat, safety glasses and gloves).
- Ensure French Press is used only with samples of plant, animal or bacterial cells (or materials described in manufacturer's instructions).
- Do not over-pressurize the cells: Before applying pressure to the cell, determine the gauge pressure required to produce the desired pressure in the cell. The maximum working pressure with the cell currently available is 40,000 psi. This corresponds to a maximum gauge reading on HIGH of 2,520 psi. **DO NOT EXCEED THIS VALUE FOR ANY REASON.**
- If the maximum working pressure is exceeded, the seals and/or cell itself could rupture causing serious injury to the operator. Use the desired pressure required for the experiment (i.e. E. Coli only requires 10,000 psi for efficient lysis).

**Notes:**

- Use of the French Press is limited to members of the Department of Molecular and Cellular Biology, unless written permission is provided by a Departmental Chair. Contact Jamie Jones (SCIE 4482 ext. 53816) to obtain information on this process. Users must take responsibility for any damage incurred to the instrument.
- Over-tightening the pressure screw/flow valve will force the nylon ball out of shape causing poor cell lysis and equipment damage in addition, stripping of the screw threading. The sample outlet tube is very delicate, please attach only during use.
- Review the manufacturer's instructions prior to using equipment.

**Procedure:**

1. Sign out pressure cell components from Chemistry Stores (Nylon balls, outlet hose) in SCIE 1110. The actual pressure cell is stored in 4°C in SCIE 3202A.
2. Check the condition of the O-rings on the piston and closure plug before and after each use. If piston does not go smoothly into the cell body, apply a small amount of vacuum grease to all O-Rings.
3. Place the piston into the top opening of the French pressure cell to the desired depth depending on your sample volume. (Min. volume of 5mL, Max. volume of 25mL). Place the cell on the filling stand upside down (text upside down). See Figure 1:
4. Fill the cell with sample to a level that will cause a small amount of it to be forced through the sample outlet tube when the closure plug is inserted. Approximately ¼" from the top. When placing closure plug into cell body, slightly pull piston back to allow your entire sample to enter the cell body and minimize sample loss due to spillage.
5. Check the white nylon ball at the end of the flow valve (make sure it is securely in place), replace if deformed (this ball helps to regulate the flow of sample through the outlet hose). Attach the outlet hose and flow valve to the closure plug and modestly tighten the flow valve (finger tighten).
6. Open the flow valve, insert the closure plug into the cell, and push down until your sample begins to enter the outlet hose. This action forces out all the air from the cylinder, and drains excess sample through the sample outlet tube. Close the flow valve by tightening modestly (finger tighten).
7. With one hand, support the bottom of the closure plug whilst the other hand is supporting the cell body tightly together. Invert the assembled unit (piston on top, closure plug on bottom), and place on the French Press. Piston arms should be perpendicular to the fastening latch. The outlet hose and flow valve should face forward.



**WARNING: ENSURE THAT THE T-HANDLE ON THE PRESSURE CELL PISTON IS ALIGNED PERPENDICULAR TO THE CELL CLAMP THUMBSCREWS. FAILURE TO PROPERLY ALIGN THE PISTON CAN CAUSE THE PISTON HANDLE AND THUMB SCREWS TO MEET, BENDING THE HANDLE AND POSSIBLY CAUSING THE THUMBSCREWS TO BREAK AND BECOME AIRBORNE.**

8. Tighten the screws that hold the cell in place.
9. Place end of outlet hose into your collection vessel.
10. **ENSURE ADJUSTMENT LEVER IS IN THE DOWN POSITION, AND THE PRESSURE ADJUSTMENT KNOB IS TURNED FULLY COUNTER CLOCKWISE.**
11. Turn the press ON.
12. Set the adjustment lever to MEDIUM, and turn the pressure adjustment knob clockwise until the gauge reads 300. Allow 10 seconds for pressure to equalize at 300 or until the piston reaches the top of the French Press. (10,000 psi or 640 on the gauge is sufficient for bacterial lysis).
13. Turn pressure adjustment knob clockwise until the gauge reads 1000.

**NOTE – DO NOT EXCEED THE PRESSURE LIMIT OF THE CELL IN USE.**

14. Turn adjustment lever to HIGH for 40 K cell only, mini cell set to MED only. **DO NOT USE THE HIGH SETTING FOR THE MINI CELL.**
15. Sample should begin to drain from the outlet hose. Recheck psi reading on the pressure gauge to ensure the predetermined pressure value. Adjust the flow by gently turning the flow valve. A flow of 3-4 drops/second is recommended. The flow valve is very sensitive, turn slowly in light increments.
16. When the entire sample has passed through the cell, release the pressure in the cell by completely opening the flow valve, then turn the adjustment lever to the down position and allow stand to reach 1 cm above the French Press body. **TURN THE PRESSURE ADJUSTMENT KNOB FULLY COUNTER CLOCK-WISE.**
17. Turn the Press OFF.
18. Complete mechanical lysis may require more than a single pass through the press. To repeat a run with the same sample, return to step 3. There may be 1-2 ml of volume loss per pass.
19. When finished, thoroughly clean the cell and all accessories with 70% ethanol, followed by a wash with distilled water using kimwipes. Do not use paper towels or abrasive cleaners.
20. Document usage in the logbook provided.
21. Allow cell to dry thoroughly at room temperature. Once dry, return outlet tubing and flow valve to Chemistry Stores SCIE 1110 and return pressure cell to the 4°C.

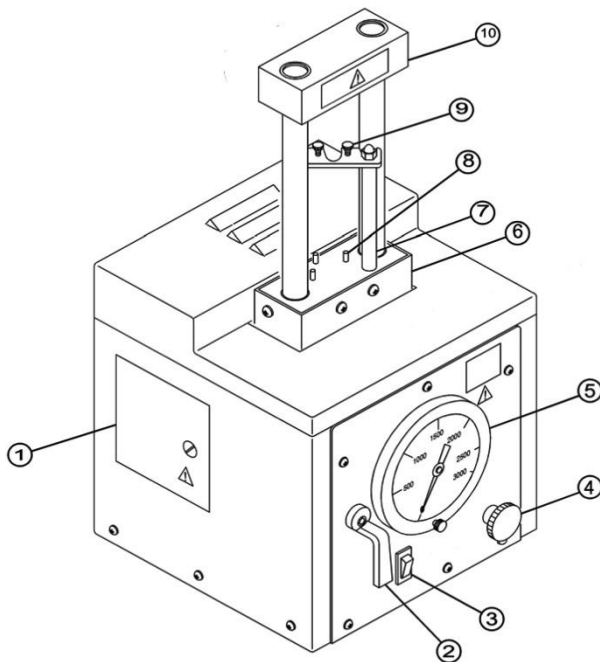
**French Pressure Cell Gauge settings and pressure limits.**

CELL PRESSURE	GAUGE PRESSURE		
	PISTON 1" DIA (40k Cell)		PISTON 3/8" DIA. (Mini Cell)
	MED RATIO	HIGH RATIO	MED RATIO ONLY
500	220		80
1000	400		100
2000	720	140	140
3000	1050	200	190
4000	1380	270	240
6000		400	340
8000		520	430
10,000		640	520
12,000		770	600
14,000		900	700
16,000		1020	800
18,000		1150	900
20,000		1280	1000
25,000		1580	
30,000		1900	
35,000		2200	
40,000		2500	

**Figure 3: Description of indicators and controls (Ref: Thermo IEC Operation Manual: OMFA078A Revision 0).**

1. **Side Panel Access Door:** Opens to gain access to hydraulic fluid reservoir inside the press.
2. **Ratio Selector Valve:** MED or HIGH position is used in conjunction with PRESSURE INCREASE CONTROL to control pressure against lower platen (item 7). DOWN position permits lower platen to descend for pressure cell installation or removal.
3. **PUMP Switch:** Controls application of AC power to pump motor inside unit.
4. **PRESSURE INCREASE control:** Used in conjunction with RATIO SELECTOR control lever to regulate amount of pressure applied to lower platen (item 7). Clockwise rotation increases pressure, while counter clockwise rotation decreases pressure.
5. **Pressure gauge, 0-3000 psi:** Indicates pressure value at RATIO SELECTOR valve. The actual pressure value applied to lower platen depends upon the position of RATIO SELECTOR lever (HIGH, MED or DOWN).
6. **Lower platen:** Moves up or down to permit cell installation and subsequent pressurization to selected psi value.

Three alignment pins on lower platen are used to position lower section of cell body in press.



7. **Cell clamp/Support Rods (2 required):** Provides support for the cell clamp. Remove rods, install rod extensions, replace rods for use with the 40K Cell.
8. **Aligning Pins (3 required):** Centers the cell in the press.
9. **Cell Clamp with thumb screws:** Locks top of pressure cell in position to ensure proper cell alignment during pressurization.
10. **Upper Platen:** Provides a mechanical stop for the top of the pressure cell piston. Used also in conjunction with the spacer and piston retainer (not supplied) for positioning and housing these components when the rapid-fill pressure cells are used in the press.
11. **Line cord:** Inputs AC power to the unit.

**Additional Figures: Reference: Thermo IEC Operation Manual: OMFA078A Revision 0**

Figure 1: 40 K Pressure Cell in filling stand.

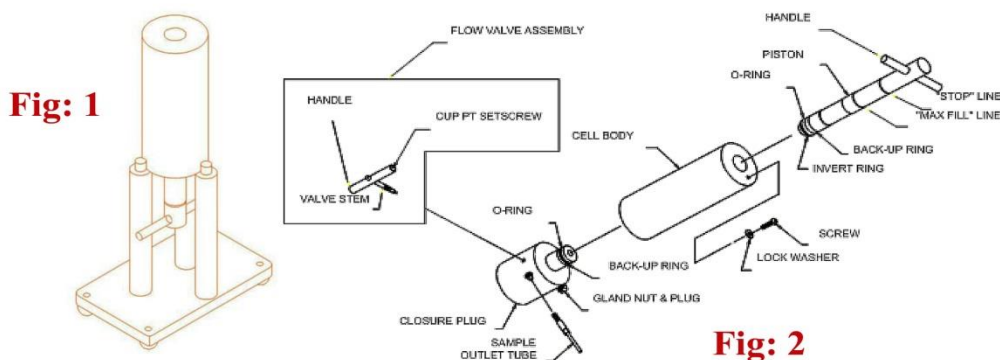
Figure 2: Exploded diagram of the 40K manual-fill assembly.

Figure 3: Controls and indicators of the French Pressure Cell Press

Figure 4: Photo of the IEC French Press

Figure 5: 40 K and Mini Pressure Cells

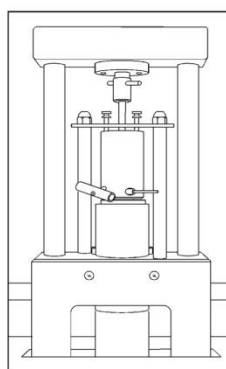
Figure 6: The Mini-cell



**Fig: 4**



**Fig: 5**



**Fig: 6**