

SOP: SP027.1
Modified 4/28/17 by MCL

Operation of French Press

Materials and Reagents:

1. Whole cells (note 1)
2. Breaking buffer (note 2)
3. Ice bucket
4. Ice
5. Glycerol
6. French Press cell (note 3)
7. French Press cell stand
8. Falcon centrifuge bottle, 225 ml
9. Disinfectant solution
10. Ethanol solution, 70%
11. Transfer pipet
12. Paper towels

Protocol:

1. _____ Suspend cells in breaking buffer at a concentration of 2 g/ml (note 4).
2. _____ Place suspended cells on ice.
3. _____ Use a small amount of glycerol to lubricate French Press piston and base o-rings (note 5).
4. _____ Assemble the pressure cell base by attaching the plug (with nut), sample outlet tube, and pressure release knob (note 6).
5. _____ Place French Press piston into unit until reaching “max fill” mark.
6. _____ Turn unit upside-down and place on French Press stand.
7. _____ Add cell suspension to French Press unit, leaving enough room to attach bottom to the unit.
8. _____ Loosen the pressure release valve to allow excess air to escape, and attach bottom to unit.
9. _____ Keeping a tight hold on the main cell unit and the base, turn the complete unit right-side up and place in French Press (notes 7 and 8). When the cell is placed in the French Press, be sure that the unit is flush against the bottom pegs, and that the piston handle is perpendicular to the bracing bar.
10. _____ Move lever to the “High” setting (“Med” setting if using the mini-cell) and turn the French Press on.
11. _____ Making small adjustments using the pressure release knob, keep pressure on cell between 1000 and 1500 while collecting eluent into the 225ml Falcon centrifuge bottle (note 9).
12. _____ When French Press cell is empty, turn machine lever to “Down”.
13. _____ Place eluent collection bottle on ice, and carefully remove cell from the French Press.
14. _____ Repeat steps 6 to 13 until the entire cell suspension has been passed through the French Press cell a total of six times.
15. _____ Smear a small amount of cell paste on a glass slide and check breakage by acid fast staining (note 10).

16. _____ Thoroughly clean French Press cell by completely disassembling unit, and washing each part with disinfectant, distilled water, then 70% ethanol (note 11).
17. _____ Allow all parts to completely air-dry prior to storage.

Notes:

1. *M. tuberculosis* cells must be γ -irradiated according to SOP PP004 prior to breaking.
2. Breaking buffer is made according to SOP PP007, PP008 or PP015, depending on your downstream needs.
3. French Press cell unit contains the following parts: French Press cell, piston, base, plug (with nut), pressure release valve, and sample outlet tube. The French Press Mini-Cell will also need a silicone bead on the pressure release valve.
4. Cell solution should be viscous in order for proper breaking to occur. If the sample becomes too thin, it can be placed briefly at -80°C .
5. Both the base and piston are equipped with dual o-rings. Be sure that the back-up o-ring is installed correctly with the concave side facing the primary o-ring.
6. The nut holding the plug in place should be tightened with a wrench. All other fittings should be hand-tightened only in order to avoid damage.
7. Care should be taken to hold onto the unit base to prevent sample loss due to the bottom falling off.
8. If the unit is over-filled, or if there is air trapped in unit, the piston may sit too high for the unit to fit properly in the French Press. In some cases, applying pressure by hand may be enough to force the piston down. If this is not successful, remove the unit and place back on the stand. Remove the base and pipet a small amount of cells out before trying again. **DO NOT ATTEMPT TO REPOSITION THE PISTON WHILE THE UNIT IS UPSIDE DOWN CONTAINING SAMPLE. THIS IS ONE OF THE MOST COMMON CAUSES OF SPILLS.**
9. Care should be taken to point mouth of bottle away from face, as small air pockets inside the French Press cell may cause unpredictable eruption of eluent when expelled.
10. See SOP SP035 for Acid-Fast staining. Cells should be more than 90% broken. Lysate will appear as a field of blue debris with few pink bacilli. If cells are not adequately broken, pass back over the French Press until sufficient cell disruption is achieved.
11. The French Press cell should be cleaned immediately after use to prevent cell debris from drying and causing clogs in the system.

Reference:

Thermo IEC Operation Manual OMFA 078A Revision 0