French Press Cell Lysis

Materials

Recombinant *E. coli* BL21(DE3) cultures
LB medium
Ampicillin
IPTG
Ice-cold phosphate-buffered saline (PBS)
Refrigerated centrifuge
High-pressure homogenizer or French press
Centrifuge tubes
Ice

Procedure

1. Prepare an overnight culture for each recombinant *E. coli* BL21(DE3) strain in LB medium containing ampicillin.
2. Measure the OD_{600} of each overnight culture before inoculation.
3. Inoculate each overnight culture into 15 mL LB medium containing ampicillin. Adjust the inoculation volume according to the OD_{600} of the overnight culture using the following relationship:

   $$Inoculation volume\ (\mu L) = \frac{6}{OD_{600}}$$

   For example, an overnight culture with an OD600 of 0.6 is inoculated at 10 μL into 15 mL LB, whereas a culture with an OD600 of 1.2 is inoculated at 5 μL.
4. Incubate the inoculated cultures at 37 ° C with shaking for 8 h.
5. Add IPTG to each culture to a final concentration of 0.4 mM.
6. Continue incubation at 37 ° C with shaking for 4 h to induce recombinant protein expression.
7. Transfer the cultures into centrifuge tubes and centrifuge at 5,000 × g for 15 min at 4 ° C.
8. Discard the culture supernatant and retain the cell pellet.
9. Add 2 mL ice-cold PBS to each pellet and resuspend the cells thoroughly.
10. Centrifuge the resuspended cells at 5,000 × g for 10 min at 4 ° C.
11. Discard the PBS supernatant. This constitutes the single PBS-washing step.
12. Resuspend each washed cell pellet in 2 mL ice-cold PBS.
13. Keep the cell suspensions on ice before cell disruption.
14. Disrupt the cells using a precooled high-pressure homogenizer or French press at a pressure of approximately 11-13 kpsi. Apply the same pressure conditions to all experimental groups.
15. Keep the resulting lysates on ice throughout subsequent handling.
16. Centrifuge the lysates at 12,000 × g for 60 min at 4 ° C.
17. Carefully collect the supernatant as the soluble protein fraction.

18. Retain the pellet as the insoluble protein fraction and resuspend it in 2 mL PBS.
19. Reserve aliquots of both the soluble and insoluble fractions for subsequent SDS-PAGE analysis.