

Bugbusting Protein Expression

Grow and induce *E. coli* as per the specific protocol. Pellet the cells by centrifuging at 4200 rpm for 20 min. Pour off supernatant, leaving 10 ml in tube. Resuspend pellet in this, transfer to a 50 ml Falcon tube and centrifuge at 3750 rpm for 10 min. Pour off the supernatant and freeze pellet in liquid nitrogen. Store at -80°C until ready to use.

Thaw pellet and weigh. Make up stock solution of Bugbuster and benzonase (add 25 units per ml of Bugbuster). Add 5 ml of Bugbuster per gram of pellet and vortex to resuspend cells. Incubate on shaking platform for 20 mins at room temperature. Sonicate for 1 min and then centrifuge at 12,000 rpm for 10-15 mins to clear the lysate. Continue with appropriate purification method.