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Protein Extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.bjdnki5e

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The four Bacillus bacteria were inoculated in 200 ml LB medium (Serva) and incubated at 37ºC overnight. The cells were centrifuged at 13000 rpm for 15 minutes at 4°C to pellet the cells. The cell pellet was sonicated at amplitude 30%, pulse 10 seconds, gap 10 seconds, for a total time of 2 minutes: 30 seconds or 15 Cycles to obtain the cell lysate. The TRIzol extraction protocol was used with minor modifications(24). In brief, to the cell lysate, 1 mL of TRIzol reagent and 200 µL of chloroform were added. The solution was mixed vigorously and was centrifuged 14,000g for 15 minutes at 4°C. The Upper clear phase was carefully decanted and to the remaining phase, 300 µL of ethanol was added and incubated at room temperature for 3 minutes followed by centrifugation at 2000g for 15 minutes at 4 °C. To the supernatant, a 4-fold volume of ice-cold acetone was added and kept for overnight incubation at -20ºC. The protein pellet was washed thrice first with 0.3 M Guanidium-HCl in 95 % ethanol followed by ice-cold acetone two times. All the protein pellets were air dried and wer subsequently dissolved in rehydration buffer (7 M urea, 2 M thiourea, 2 % CHAPS, Distilled water). Protein concentrations were estimated using Bradfor assay.