Protein Expression and Purification

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Abstract

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Protocol

The plasmids (His-tagged constructions) were transformed into BL21(DE3)pLysS or Rosetta(DE3)pLysS E. coli cells (Novagen).

Step 1.

Colonies were inoculated into 20 ml LB medium containing 100 µg/ml ampicillin and 34 µg/ml chloramphenicol and grown overnight at 37 °C with shaking.

Step 2.

The culture was then transferred into 1 L of fresh LB medium containing 100 µg/ml ampicillin and 34 µg/ml chloramphenicol.

Step 3.

When the culture was grown to OD600=0.5-0.6 at 37 °C with shaking (250 rpm), protein expression was induced with 0.6 or 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). At the same time, 100 mg/L FeSO4·7H2O and 17 mg/L δ-aminolevulinic acid hydrochloride (ALA) was also added to enhance heme biosynthesis.

Step 4.

The culture was further incubated at 37 °C for 3-4 hours or 30 °C for 6-8 hours.

Step 5.

Cells were harvested by centrifugation at 5000 rpm and stored at -20 °C prior to purification.

Step 6.

A cell pellet from 2 L of culture was resuspended in 20 ml of buffer I (200 mM NaCl, 50 mM Na2HPO4, pH 8.0) containing 1mM phenylmethylsulfonyl fluoride (PMSF) and sonicated for 8 min (24 pulses of 20 s with 20 s pauses, Sonic Dismembrator 550, Fisher Scientific).

Step 7.

The cell lysate was centrifuged to remove the insoluble cell debris, and the supernatant was filtered (0.22 µm pore size, Corning) and kept on ice until loaded onto the purification column.

Step 8.

The BioCAD SPRINT perfusion system (Applied Biosystems) was used for metal affinity chromatography. The column (POROS MC/20, 10 × 100 mm, column volume 7.9 ml) of immobilized Co2+ resin was prepared by first washing with five column volumes (CV) of 50 mM EDTA, 1 M NaCl (pH 8.0) and rinsing with an equal volume of distilled water.

Step 9.

The metal was loaded with 5 CV of 100 mM CoCl2·6H2O, washed with 5 CV of water, and followed by 5 CV of 3 M NaCl to remove residual metal ions.

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Step 10.

A flow rate of 15 ml/min was used for all the aforementioned washes.

Step 11.

Prior to sample loading, the column was washed with 5 CV of buffer I containing 0.5 mM imidazole (flow rate 8.0 ml/min). The sample was loaded onto the column at a flow rate of 0.5-1.0 ml/min, followed by 6-8 CV of buffer I at 2.0 ml/min.

Step 12.

The column was then washed with 8 CV of buffer I containing 0.5 mM imidazole (5.0 ml/min) to remove non-specifically bound proteins.

Step 13.

The remaining bound proteins were eluted by a linear gradient of imidazole from 0.5 mM to 250 Mm in buffer I (5.0 ml/min flow rate, 7 CV total), and collected in 1.5 ml fractions.

Step 14.

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