Sep 27, 2016 Version 1

Protein Expression & Isolation & Purification V.1

DOI
dx.doi.org/10.17504/protocols.io.fwvbpe6

Caroline Green

Caroline Green
Creative BioMart

DOI: dx.doi.org/10.17504/protocols.io.fwvbpe6

Protocol Citation: Caroline Green 2016. Protein Expression & Isolation & Purification. **protocols.io https://dx.doi.org/10.17504/protocols.io.fwvbpe6**

Protocol status: Working

Created: September 27, 2016

Last Modified: March 27, 2018

Protocol Integer ID: 3765

1 **Object**

- To know the cloned gene expression method and meaning;
- To learn recombinant protein purified by **affinity chromatography**;

Principle

Cloned gene expression in cells are of great significance both for theoretical and experimental applications. Through expression, we can explore the function and the mechanism of regulation of gene expression, as well as the protein encoded by cloned gene expression can be used for the study of structure and function. E. Coli is the most widely used **protein expression** systems and the expression level of exogenous gene product is much higher than other gene expression systems. The amount of targeted protein expressed is even more than 80% of the total bacterial proteins.

In this study, a plasmid carrying the gene of the target protein in E. coli BL21, and at 37 ℃, IPTG induced overexpression of carries six consecutive histidine residues recombinant chloramphenicol acetyltransferase protein that available one kind by covalent conjugation of nitrilotriacetic acid (NTA) nickel ions (Ni2 +) immobilized chromatographic media to be purified, in fact boil metal affinity chromatography (MCAC). The degree of purification of the protein can be analyzed by polyacrylamide gel electrophoresis.

Reagents and Equipment

Reagent

- LB broth: Trytone 10g, yeast extract 5g, NaCl 10g; add up to 1000ml with distilled water;
- ampicillin: 100mg / mL
- Sample

Buffer: 100 mM NaH2PO4, 10 mMTris, 8M Urea, 10 mM2-ME, pH8.0
(4) Washing Buffer : 100 mM NaH2PO4, 10 mM Tris, 8 M Urea, pH6.3
(5) Elution Buffer:100 mM NaH2PO4, 10 mMTris, 8M Urea, 500 mM Imidazole, pH8.0
(6) IPTG

Equipment

Shaker, centrifuges, chromatography column (1'10 cm)

Operating method

Induction of Chloramphenicol acetyltransferase **recombinant protein**

1. Inoculate E. coli BL21 strain containing the recombinant protein of chloramphenicol acetyltransferase in 5mL LB liquid medium (containing 100ug/mL ampicillin), 37 ℃ shaking and culture overnight.
2. Transfer 1mL overnight cultures in 100 mL (containing 100ug/mL ampicillin) LB liquid medium; 37 ℃ shake culture until OD600 = 0.6 - 0.8. Take 10ul samples for SDS-PAGE

analysis.

3. Add IPTG to a final concentration of 0.5 mmol/l, culture 1-3h at 37 ℃.
4. 12,000rpm centrifugation for 10 min, discard the supernatant; store cell precipitate at -20 ℃ or -70 ℃.

To be continued...