- Superior alternative to BL21(DE3) for routine protein expression
- Improved purity of target proteins isolated by IMAC
- Identical growth characteristics as BL21(DE3)
- Transformation efficiency: 1–5 x 10^7 cfu/µg pUC19
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (*fhuA2*)
- Free of animal products

Description:

Chemically competent *E. coli* cells derived from BL21(DE3). Poly-histidine tagged recombinant proteins that are isolated by immobilized metal affinity chromatography (IMAC) are often contaminated with significant amounts of endogenous *E. coli* metal binding proteins. The protein expression strain NiCo21(DE3) has been engineered to minimize *E. coli* protein contamination of IMAC fractions: GlmS is mutated to eliminate binding to IMAC resins and three other proteins (SlyD, ArnA and Can) are tagged to enable rapid removal by chitin affinity chromatography.

Reagents Supplied:

20 x 0.05 ml/tube of chemically competent NiCo21(DE3)
Competent *E. coli* cells **(Store at -80°C)**
20 ml of SOC Outgrowth Medium **(Store at Room Temperature)**
0.025 ml of 50 µg/ml pUC19 Control DNA **(Store at -20°C)**

Compatible expression vectors/promoters

T7 or T7-lac promoter, P_{tac}, $P_{trc,}$ P_{lac}, $Plac_{UV5}$, ParaBAD vectors, PrhaBAD.
Note: Expression from vectors containing a T5-lacO promoter will not be regulated unless the expression vector also encodes the lacI gene or a compatible vector expresses LacI.

Genotype:

can::CBD fhuA2 [lon] ompT gal (λ DE3) [dcm] arnA::CBD slyD::CBD glmS6Ala ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5

Transformation Protocol Variables

Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes you shorten this step.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 10 seconds at 42°C is optimal.

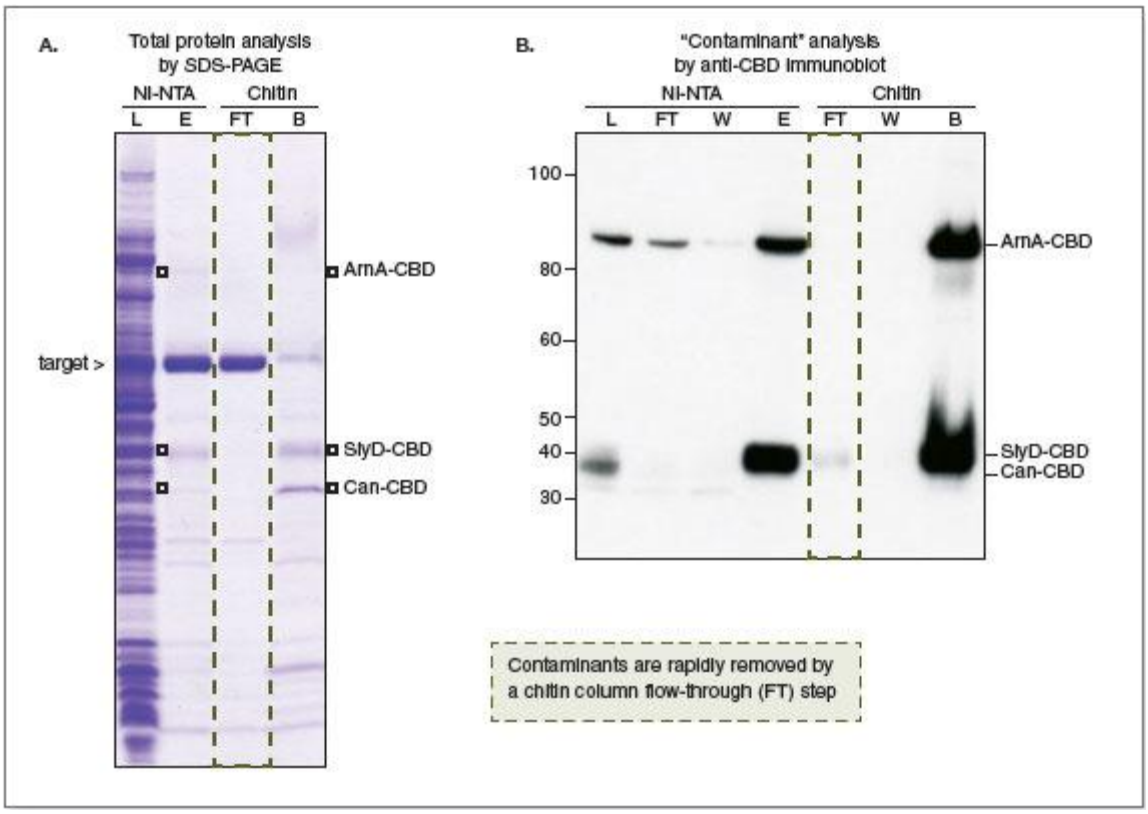
Outgrowth: Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes you shorten this step. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold

higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.

Antibiotics for Plasmid Selection

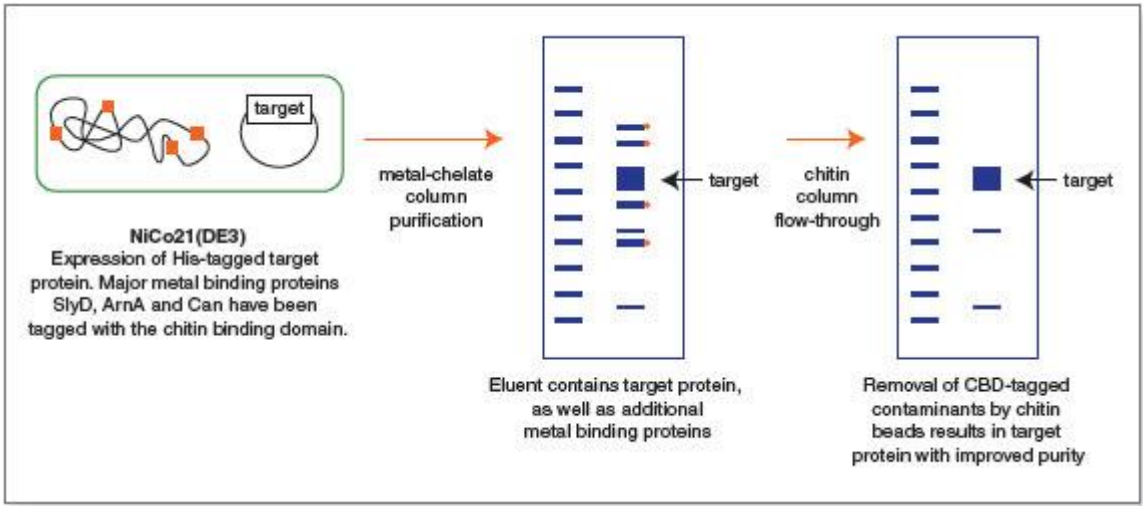
Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml



Improved purity of His-tagged proteins with NiCo21(DE3)

A) Expression of Glutamyl tRNA Synthetase (6-His) in NiCo21(DE3) Competent *E. coli* followed by Ni-NTA purification. Eluent (E) from a Ni-NTA column was passed over a chitin column. The protein of interest elutes in

the flow through (FT), while the CBD-tagged metal binding proteins remain bound (B) to the chitin resin (NEB #S6651S). Purifications were performed according to manufacturers' recommended conditions. B) Contaminants ArnA, SlyD and Can are confirmed by Western blot using Anti-CBD Antibody (NEB #E8034S).



Two-step purification of target protein that has been expressed in the NiCo21(DE3) strain of *E. coli*

Reaction & Storage Conditions

Storage Temperature:

-80°C

Notes

Usage notes:

1. **CAUTION:** This product contains DMSO, a hazardous material. Review the MSDS before handling.
2. **STORAGE AND HANDLING:** Competent cells should be stored at -80°C. Storage at -20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above -80°C, even if they do not thaw.