NiCo21(DE3) Competent *E. coli*

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C2529H

20 x 0.05 ml/tube **Lot: 0081411**

Store at –80°C

CAUTION: This product contains DMSO, a hazardous material. Review the MSDS before handling.

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.

Features:

- 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

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.

Quality Control Assays

Quality Control for Current Lot: Quality control values for a specific lot can be found on the datacard which accompanies each product.

Transformation Efficiency: 100 pg of pUC19 plasmid DNA was used to transform one tube of NiCo21(DE3) Competent *E. coli* following the protocol provided. 1–5 x 10^7 colonies formed/µg after an overnight incubation on LB-ampicillin plates at 37°C.

Untransformed cells were also tested for resistance to phage ϕ80, a standard test for resistance to phage T1 and sensitivity to ampicillin, chloramphenicol, kanamycin, nitrofurantoin, spectinomycin, streptomycin and tetracycline.

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.

High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of NiCo21(DE3) Competent *E. coli* cells on ice for 10 minutes.
2. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. **Do not vortex.**
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 10 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 20–24 hours or at 25°C for 48 hours.

5 Minute Transformation Protocol

A shortened transformation protocol resulting in approximately 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable. Follow the High Efficiency Transformation Protocol above with the following changes:

1. Steps 3 and 5 are reduced to 2 minutes.
2. Omit outgrowth (Step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Protocol for Protein Expression Using NiCo21(DE3)

1. Transform expression plasmid into NiCo21(DE3). Plate on antibiotic selection plates and incubate overnight at 37°C.
2. Resuspend a single colony in liquid culture with antibiotic to produce a starter culture. Inoculate starter culture at a 1:100 dilution into expression media containing antibiotic.
3. Incubate at 37°C with shaking until OD_{600} reaches 0.4–0.8.
4. For most vector systems, induce expression with 40 or 400 µM IPTG for 3 hours at 37°C, 5 hours at 30°C or overnight at 16°C or 23°C.

Protocol for Removal of IMAC Contaminating Proteins:

E. coli SlyD, ArnA, and Can (carbonic anhydrase) are tagged with the chitin binding domain (CBD). Accordingly, these proteins may be removed by incubating the *E. coli* lysate or the IMAC elution fractions with chitin beads (NEB #S6651) or chitin magnetic beads (NEB #E8036). Binding of CBD-tagged proteins to chitin resin is compatible in a wide range of buffer conditions. Pooled IMAC fractions may be directly mixed with buffer-equilibrated chitin beads and incubated for 5–30 minutes to remove CBD-tagged contaminants from the His-tagged target protein.

The following procedure is recommended:
Use 1 ml of chitin resin for each volume of lysate or IMAC pool corresponding to 1 gram of NiCo21(DE3) cell pellet. (or use 1 ml of chitin resin for every 100 ml of expression culture). Resuspend chitin slurry (stored in 20% ethanol) and transfer to a gravity flow column. Equilbrate chitin column with buffer similar or equivalent to the IMAC low imidazole buffer: (or use a buffer compatible with the downstream chromatography step). Seal bottom of chitin column and add cell lysate or IMAC fractions containing CBD-tagged contaminants. Seal top of column and mix by rocking for 5–30 minutes at 4°C. Elute void volume containing target protein by gravity flow and optionally add extra equilibration buffer to displace all buffer containing the target protein.

(Alternatively, if using a mini-spin column incubate sample 5–30 minutes before centrifuging to elute the target protein).

Analyze eluted protein by SDS-PAGE or Western blot to determine purity. Removal of CBD-tagged contaminants may be analyzed by Anti-CBD Monoclonal Antibody (NEB #E8034).

(see other side)

CERTIFICATE OF ANALYSIS

Troubleshooting T7 Protein Expression

No colonies or no growth in liquid culture: This outcome is often due to basal expression of a target gene product, which is detrimental to cell viability. Basal expression of target protein in NiCo21(DE3) is identical to basal expression in BL21(DE3). Some vector systems (in particular those utilizing a T7 promoter) allow expression without inducer. If tightly regulated T7 expression is required, use a strain expressing *lysY:*

- T7 Express *lysY* (NEB #C3010) *lysY* produces a mutant T7 lysozyme which binds T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA polymerase titrates out the lysozyme and results in expression of the target protein.
- T7 Express *lysY/Iq* (NEB #C3013) *lysY* expression as well as *lacI* over-expression to repress basal expression of the T7 RNA polymerase.
- Lemo21(DE3) (NEB #C2528) BL21(DE3) containing the Lemo System™. LysY expression is modulated by L-rhamnose, making T7 protein expression tightly regulated and tunable.

No protein visible by SDS-PAGE or no activity:

- Check for toxicity – no protein may mean the cells have lost the expression plasmid or elements of the expression plasmid have been deleted.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, there will be a significant difference between the number of colonies on the plates. Fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic. If the expression plasmid is not maintained by drug selection, *lysY* strains will improve clone stability.
- Check clone integrity by restriction enzyme analysis and/or sequencing of the ORF.

Induced protein is insoluble: T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. Potential solutions are:

- Induce at a lower temperature (as low as 15°C overnight)
- Reduce IPTG concentration to 10–40 µM
- Induce earlier in growth phase (OD_{600} = 0.3 or 0.4) and harvest cells earlier.
- Create an expression construct where the target protein is fused to Maltose Binding Protein (NEB #E8200).

Solutions/Recipes

SOB:

2%	Vegetable peptone (or Tryptone)
0.5%	Yeast Extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgCl_2$
10 mM	$MgSO_4$

SOC:

SOB + 20 mM Glucose

LB agar:

1%	Tryptone
0.5%	Yeast extract
0.17 M	NaCl
1.5%	Agar

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

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.

Strain Properties

The properties of this strain that contribute to its usefulness as a protein expression strain are described below.

T7 RNA Polymerase: *T7 gene1* is encoded by the lambda DE3 prophage present within the chromosome. T7 RNA polymerase is expressed from the *lacUV5* promoter, which is less sensitive to catabolite repression than the wt *lac* promoter. Thus DE3 strains may exhibit uninduced target protein expression. Although λ DE3 is normally dormant in the host chromosome, the induction of the SOS response can occur as the result of expressing proteins that damage the *E. coli* chromosome, either directly or indirectly. This may lead to cell lysis. T7 Express strains do not carry the DE3 prophage and better tolerate an SOS response.

Protease Deficient ([*lon*] *ompT*): *E. coli* B strains are "naturally" deficient in the Lon protease which in K-12 strains serves to degrade misfolded proteins and to prevent some cell cycle-specific proteins from accumulating. The OmpT protease resides at the surface of wild type *E. coli* in both K-12 and B strains, presumably helping the cells to derive amino acids from their external environment. Cells deficient in both these proteases are much more amenable to the production of proteins from cloned genes.

T1 Phage Resistant (*fhuA2*): T1, an extremely virulent phage requires the *E. coli* ferric hydroxamate uptake receptor for infectivity. Deletion of this gene confers resistance to this type of phage, but does not significantly affect the transformation or growth characteristics of the cell.

glmS6Ala: The *glmS* gene (glucosamine synthetase) is mutated and the expressed GlmS protein (67 kDa) contains six histidine to alanine substitutions (positions 62, 65, 432, 436, 466, 467). The mutated GlmS protein does not bind Ni-NTA resin in the presence of 20mM imidazole binding/ wash buffer, whereas wt GlmS protein binds Ni-NTA resin and is not eluted until the imidazole concentration is within 55–80 mM (1).

arnA::CBD The arnA gene (lipid A modification) is fused with a *B. circulans* ORF encoding a chitin binding domain. The expressed fusion protein is 82 kDa whereas the wt ArnA protein is 74 kDa.

can::CBD The *can* gene (carbonic anydrase) is fused with a *B. circulans* ORF encoding a chitin binding domain. The expressed fusion protein is 32 kDa whereas wt carbonic anhydrase is 25 kDa.

slyD::CBD The *slyD* gene (FKBP-type prolyl isomerase) is fused with a *B. circulans* ORF encoding a chitin binding domain. The expressed fusion protein is 28 kDa. However, the apparent mass of the CBD fusion is approximately 35 kDa when analyzed by SDS-PAGE. The calculated mass of wt SlyD is 21 kDa, whereas the apparent mass is 28 kDa.

The GlmS(6Ala) protein and the CBD-tagged proteins are functional *in vivo* according to assays conducted at New England Biolabs.

Reference:

1. Bolanos-Garcia and Davies (2006) *Biochimica et Biophysica Acta* 1760, 1304–1313.