# High Efficiency Transformation Protocol (C2529)

## Introduction

Perform steps 1–7 in the tube provided.

## Protocol

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 (C2529)

## Introduction

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:

## Protocol

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

# Protocol for Protein Expression Using NiCo21(DE3) (C2529)

Protocol

- Transform expression plasmid into NiCo21(DE3). Plate on antibiotic selection plates and incubate overnight at 37°C.
- 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.
- Incubate at 37°C with shaking until OD600 reaches 0.4–0.8.
- 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 (C2529)

Introduction

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).