Protocol

Fold-Assist Chemically Competent E. Coli Cell Bundle
User Guide

Introduction

Fold-Assist™ Chaperone Competent Cells are *E. coli* BL21 (DE3)-based expression strains engineered to enhance the folding and solubility of recombinant proteins. Each strain carries a Fold-Assist™ (pFA series) chaperone plasmid encoding specific molecular chaperone systems that assist in protein folding during expression.

This system enables rapid screening of multiple folding environments without additional cloning steps, improving the likelihood of obtaining soluble, functional protein.

Materials

Included Strains:

- 2x 100µL BL21(DE3) + pFA-KJE-Gro — DnaK/DnaJ/GrpE + GroEL/ES
- 2x 100µL BL21(DE3) + pFA-KJE — DnaK/DnaJ/GrpE
- 2x 100µL BL21(DE3) + pFA-Gro — GroEL/ES
- 2x 100µL BL21(DE3) + pFA-GroTF — GroEL/ES + Trigger Factor
- 2x 100µL BL21(DE3) + pFA-TF — Trigger Factor

Other included supplies:

- pUC19 Control DNA, 500 pg/µl
- *E. coli* Recovery Medium (GoldBio Catalog # CC-302)

Additional materials necessary:

- Arabinose (A-300)
- Tetracycline (T-101)
- Chloramphenicol (C-104)
- LB agar selection plates
- Microcentrifuge tubes
- Shaker incubator

Storage and Handling

- This product will be shipped on dry ice. Fold-Assist Chemically Competent *E. coli* cells should be stored at -80°C, pUC19 Control DNA should be stored at -20°C and recovery medium should be stored at 4°C immediately upon arrival. When stored under the

recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.

- Thaw Fold-Assist Chemically Competent *E. coli* cells and pUC19 Control DNA ice and mix gently. After thawing, these products should be kept on ice at all times before use. These products can be refrozen for storage, though this may have a negative impact on the efficiency of future transformations.

Method

Transformation protocol

Use this procedure to transform Fold-Assist Chemically Competent *E. coli* cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. Do not use these cells for electroporation.

1. Remove competent cells from the -80°C freezer and thaw completely on ice, 5 minutes.

2. When the cells are fully thawed, tap the tube gently 4-5 times to mix, then carefully pipette 50 µl of cells to a transformation tube on ice.

   Note: Handle the competent cells gently as they are highly susceptible to mechanical lysis caused by vortexing or pipetting.

3. Add 100pg-1µg (1-5µl volume) of the plasmid to be transformed to the transformation tube containing the cells. For the pUC19 control, we recommend 2 µl of 500 pg/µl DNA DNA to cells. Flick tube 4-5 times to mix. Do not vortex!

4. Incubate the cells with DNA on ice for 30 minutes.

5. After a 30-minute incubation on ice, heat shock the cells at 37°C for 45 seconds.

6. Transfer the tubes to ice for 5 minutes.

7. Add 950 µl of Recovery Medium to each tube.

8. Incubate tubes at 37°C for 1 hour at 210 rpm in a shaker incubator.

9. Spread 50 µl to 200 µl from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 12.5 µg/mL Chloramphenicol and 50-100 µg/ml ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.

10. Incubate the plates overnight at 37°C.

Alternate: 5 Minute Transformation Protocol

The following procedure is faster than our standard protocol, but results in only ~10% of the transformation efficiency as the protocol listed above.

1. Remove competent cells from the -80°C freezer and thaw on ice 1 minutes.
2. Aliquot 1-5 µl (1 pg-100 ng) of DNA to the microcentrifuge tubes.
3. Incubate the cells with DNA on ice for 2 minutes.
4. After the 2-minute ice incubation, heat shock the cells at 37°C for 45 seconds.
5. Transfer the tubes to ice for 2 minutes.
6. Add 950 µl of Recovery Medium at room temperature or any other medium of choice to each tube. Immediate spread 50 µl to 200 µl from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least once plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on al LB plate containing 12.5 µg/mL Chloramphenicol and 50-100 µg/ml ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.
7. Incubate the plates overnight at 37°C.

Chaperone Co-expression

Optimal selection of chaperone systems and culture conditions—such as media type, temperature, aeration, induction timing, inducer concentration, and expression duration—will vary depending on the target protein. The protocol below provides a general starting point; conditions should be optimized for each protein of interest.

1. Co-expression Procedure

The following example outlines a typical co-expression workflow using an ampicillin-resistant expression plasmid under control of the *lac* promoter alongside a Fold-Assist™ chaperone plasmid:

1. Construct an expression plasmid encoding the target protein for use in *E. coli*.
2. Transform Fold-Assist strain of choice with the target protein expression plasmid.
3. Inoculate transformants into LB medium at 1/100 containing:
    - Chloramphenicol (12.5 µg/mL)
    - Antibiotic of expression plasmid

4. Incubate at 37°C until A_{600} = 0.15 – 0.20
5. Induce Chaperones
    - **Inducer guidelines:**
        - Use both L-arabinose and tetracycline for combined chaperone systems (e.g., KJE + Gro)
        - Use L-arabinose alone for KJE, Gro, or TF systems
        - Use tetracycline alone for Gro + TF systems
    - Inducer Concentrations:
        - L-arabinose: 0.5–4 mg/mL
        - Tetracycline: 1–10 ng/mL

***Recommended starting conditions**:* 0.8 mg/mL L-arabinose and/or 2 ng/mL tetracycline. Low tetracycline concentrations typically do not inhibit cell growth.

6. Decrease incubator temperature to 30°C. Induce chaperones for 1hr.
7. After 1hr, induce target protein expression. Continue incubation at 30-37°C for 1–4 hours, up to overnight.
8. After induction, evaluate protein expression and solubility using SDS-PAGE and/or activity assays. Further optimize conditions—including temperature, induction timing, inducer concentrations, and culture duration—to maximize soluble protein yield.

Associated Products

- *E. coli* Competent Cell Recovery Medium (Catalog # CC-302)
- GB5-alpha™ Chemically Competent E. coli Cells (Catalog # CC-101)
- Ampicillin (Catalog # A-301)
- Carbenicillin (Catalog # C-103)
- IPTG (Catalog # I2481C)
- BLUEstain™ Protein ladder, 11-245 kDa (Catalog # P007)
- Tris Buffer (Catalog # T-400
- Glycine (Catalog # G-630)