SoluBL21™ Chemically Competent E. coli

AMS.C700200

Kit Components

- Competent Cells, 10 x 50 µl
- 1 x 12 mL SOC Medium
- 1 x 10 µl Control Plasmid (pUC19 Control, 500 pg/µl)

Shipping: Shipped on Dry Ice

Storage: Store the SoluBL21 kit at -70°C. The SOC Medium may be stored at 4 °C. Stable for 6 months.

Introduction: The low cost and convenience of expressing mammalian proteins in E. coli make this host bacterium an important tool for life science applications. However, a major obstacle faced by scientists using E. coli expression strains, such as BL21(DE3), is the high percentage of mammalian proteins that are expressed in an insoluble form. Different approaches to dealing with protein insolubility in E. coli, such as lowering expression temperature, changing promoters, adding purification tags, using alternative media, or protein re-folding work only in some cases and at a high cost in time, effort, and complications. With the SoluBL21 Competent E. coli, we have used a novel directed evolution approach to create a significantly improved BL21(DE3) host strain. With this mutant strain, users will significantly improve their chances of obtaining partially or fully soluble proteins in the majority of expression experiments. Even in cases where partial solubility is achieved, users can obtain sufficient amounts of protein by simply increasing the size of their culture. With the new SoluBL21 strain, a major obstacle to soluble protein expression in E. coli has been overcome for many mammalian proteins. This significant improvement should enable users to make progress in a wide range of applications more quickly and far less expensively than in the past BL21 (DE3) Chemically Competent E. coli Cells. BL21 (DE3) chemically competent cells feature a widely used host background, a T7 expression strain, and are deficient in both Ion (1) and ompT proteases. In addition, BL21 (DE3) cells are resistant to phage T1 (fhuA2) and are B strain.

SoluBL21™ Strain: F- ompT hsdSB (rB- mB-) gal dcm (DE3)	
DE3	Encodes T7 lysogen for T7 RNA polymerase for high-level transcription
ompT	Deficient in the OmpT protease, resulting in a higher yield of intact recombinant proteins
hsd SB (rB- mB-)	Improved transformation efficiencies and representations of methylated DNA

Material needed:

- Ampicillin
- LB agar selection plates
- Microcentrifuge tubes
- Shaker incubator

Thaw BL21 (DE3) Chemically Competent E. coli cells and pUC19 Control DNA ice and mix by gentle vortexing. After thawing, these products should be kept on ice before use. These products can be refrozen for storage.

Transformation protocol

Use this procedure to transform SoluBL21 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.

Note: Handle the competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.

Note: Thaw competent cells on ice and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

1. Remove competent cells from the -80°C freezer and thaw completely on ice (10-15 minutes).

2. Aliquot 1-5 µl (1 pg-100 ng) of DNA to the chilled microcentrifuge tubes on ice.

3. When the cells are thawed, add 50 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC19 control, add 2 µl of (500 pg/µl) DNA to a chilled microcentrifuge tube, prior to adding 50 µl of cells. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.

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 10 seconds.

6. Transfer the tubes to ice for 2 minutes.

7. Add 950 µl of SOC Medium or any other medium of choice 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 100 µg/ml ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.

10. Incubate the plates overnight at 37°C.

5 Minute Transformation Protocol

The following procedure results in only ~10% of the transformation efficiency as the protocol listed above.

1. Remove competent cells from the -80°C freezer and thaw in your hand.

2. Aliquot 1-5 µl (1 pg-100 ng) of DNA to the microcentrifuge tubes. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.

3. Incubate the cells with DNA on ice for 2 minutes.

4. After the 2-minute ice incubation, heat shock the cells at 42°C for 45 seconds.

5. Transfer the tubes to ice for 2 minutes.

6. Add 950 µl of SOC 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 100 µg/ml ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.

7. Incubate the plates overnight at 37°C.

Calculations

Transformation efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1 µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Dilution

Where:

Colonies = the number of colonies counted

µg = amount of DNA transformed in µg

Dilution = total dilution of the DNA before plating

Example:

Transform 1 µl of (10 pg/µl) pUC19 control plasmid into 50 µl of cells, add 950 µl of SOC Medium. Dilute 10 µl of this in 990 µl of Recovery Medium and plate 50 µl. Count the colonies on the plate the next day. If you count 250 colonies, the TE is calculated as follows:

Colonies = 250

µg of DNA in 10 pg = 0.00001

Dilution = 10 µl/1000 x 50 µl/1000 = 0.0005

TE = 250/0.00001/0.0005 = 5.0×10^{10}

Protein Expression

1. Inoculate a colony of the SoluBL21 into 1-2 ml of M9 minimal media with appropriate antibiotic.

2. Grow overnight at room temperature in a shaking incubator at 200 rpm.

3. Dilute cells into the same media until OD600 = 0.2

NOTE: if cells are stationary, the dilution is approximately 1:20

4. Grow cells at room temperature until OD600 = 0.4. This will take approximately 90-120 minutes.

5. Add IPTG to a final concentration of 1 mM.

6. Incubate cells overnight at room temperature, in a shaking incubator at 200 rpm.

NOTE: For some clones, expression at lower temperatures may improve solubility. If the amount of soluble protein expression at room temperature is low or unsatisfactory, we recommend trying an overnight expression experiment at 20°C instead. Since each clone under investigation has different properties, you may wish to test a few basic expression conditions in small scale prior to larger scale production.

7. Spin down the cells and process as desired. For soluble protein extraction, we recommend the SoluLyse™ Protein Expression Reagents.