

SoluBL21™

Competent *E. coli* Kits



A division of Gene Therapy Systems, Inc.

| Cat. # | Contents | Quantity | Related Products | Catalog # |
|-----------------|---------------------------------------------------------------------------------------------|-------------------|-------------------------------------------------------------------|------------------------------------|
| C700200 | SoluBL21™ Chemically Competent <i>E. coli</i> | 10 x 50 µl | SoluLyse™ Bacterial Protein Extraction Reagent (Phosphate Buffer) | L100125 (125 ml); L100500 (500 ml) |
| | SOC Medium | 6.0 ml | SoluLyse™ Bacterial Protein Extraction Reagent (Tris Buffer) | L200125 (125 ml); L200500 (500 ml) |
| | pUC19 Positive Control Plasmid | 20 µl (500 pg/µl) | EZ-Spread™ Beads, Single-Use Tubes | C400050 (50 tubes) |
| C700210 | SoluBL21™ Electrocompetent <i>E. coli</i> | 10 x 20 µl | EZ-Spread™ Beads, Dispenser Bottle | C400100 (1 bottle) |
| | SOC Medium | 6.0 ml | TurboCells® Competent <i>E. coli</i> | C300020 (20 x 50 µl) |
| | pUC19 Positive Control Plasmid | 20 µl (10 pg/µl) | TurboCells® BL21(DE3) Competent <i>E. coli</i> | C302020 (20 x 50 µl) |
| | | | TurboCells® BL21(DE3) pLysS Competent <i>E. coli</i> | C303020 (20 x 50 µl) |
| | | | SmartCells™ Competent <i>E. coli</i> | C101020 (20 x 50 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*, Genlantis scientists 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 Genlantis 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.

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

[†] The SoluBL21 strain contains uncharacterized mutations obtained through special selection criteria. These mutations make the strain able to express insoluble proteins in soluble form, fully or partially, in most tests conducted.

METHODS AND PROCEDURES

A. General Notes

The SoluBL21 transformation efficiency is $\geq 10^6$ cfu/µg for chemically competent cells, and $\geq 10^{10}$ cfu/µg for Electrocompetent cells. We recommend testing efficiency by using 2 µl of the pUC19 Positive Control Plasmid per transformation reaction. Plate transformation mix on LB agar with 100 µg/ml carbenicillin.

B. Media Preparation

Protein expression in the SoluBL21 *E. coli* is optimized for use with M9 Minimal Media (M9). Prepare the M9 media as follows:

a. Mix the M9 salts (at 1X) by combining, per liter:

| | |
|----------------------------------|--------------|
| Na ₂ HPO ₄ | 6 g |
| KH ₂ PO ₄ | 3 g |
| NaCl | 0.5 g |
| NH ₄ Cl | 1 g |
| Water | up to 800 ml |

b. Filter sterilize or autoclave.

NOTE: Alternatively, make a 10X stock of M9 salts, sterilize, and store at room temperature until needed. Dilute to 1X and proceed to step c. below.

c. Add the following sterile components (per liter):

| | |
|--------------------------|-----------------------|
| 100 mM CaCl ₂ | 1 ml |
| 1 M MgSO ₄ | 1 ml |
| Glycerol | 0.3% final |
| Sterile Water | up to 1L final |

C. Chemical Transformation Protocol

1. Thaw one vial of chemically competent SoluBL21 cells on ice for a few minutes.
2. Transfer 50 μ l of cells into a sterile 15 ml snap cap tube.
3. Add 1-10 ng of plasmid DNA to the SoluBL21 cells.
4. Mix cells and DNA well, and incubate on ice for 15 minutes.
5. Heat shock the transformation mix at 42°C for 45 seconds.
6. Add 0.25 ml room temperature SOC Medium and incubate at 37°C for 1 hour in a shaking air incubator.
7. Plate the entire contents of the transformation reaction on an LB plate with appropriate antibiotic selection.
8. Incubate overnight at 37°C.

D. Electroporation Protocol

9. Place 0.1 cm cuvette on ice for at least 5 minutes. Thaw cells on ice.
10. Add 1 μ l of miniprep or ligation mix DNA directly to 20 μ l of cells
NOTE: If using a topoisomerase cloning system, follow the manufacturer's recommendations for electroporation buffer.
11. Incubate on ice 10 minutes.
12. Pipet cells + DNA into cuvette. Keep on ice.
13. Wipe cuvette free of ice and moisture and place in electroporation chamber.
14. Electroporate using 2.25 kV, 400 ohms, and 25 μ F settings.
15. Immediately add 0.4 ml SOC to chamber. Pipet up and down 5-10 times until cells are well mixed; add entire volume to a 15 ml snap cap tube.
16. Incubate 90 minutes at 37°C.
17. Plate the entire contents (or a dilution if needed) of the transformation reaction on an LB plate with appropriate antibiotic selection.

E. Protein Expression

18. Inoculate a colony of the SoluBL21 into 1-2 ml of M9 minimal media with appropriate antibiotic.
19. Grow overnight at room temperature in a shaking incubator at 200 rpm.
20. Dilute cells into the same media until OD₆₀₀ = 0.2
NOTE: if cells are stationary, the dilution is approximately 1:20
21. Grow cells at room temperature until OD₆₀₀ = 0.4. This will take approximately 90-120 minutes.
22. Add IPTG to a final concentration of 1 mM.
23. 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.
24. Spin down the cells and process as desired. For soluble protein extraction, we recommend the SoluLyse™ Protein Expression Reagents (See Related Products table above).

LIMITED LICENSE: The purchase price paid for the SoluBL21 *E. coli* Expression Kits (hereto "SoluBL21") grants end users a non-transferable, non-exclusive license to use the kits and/or their components for internal noncommercial research purposes only as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service, including proteins expressed directly through the use of the SoluBL21 cells, without the written approval or license from Genlantis, a division of Gene Therapy Systems, Inc. (GTS). Additionally:

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