SoluBL21™

Competent *E. coli* Kits

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
C700200	SoluBL21™ Chemically Competent *E. coli*	10 x 50 µl
	SOC Medium	6.0 ml
	pUC19 Positive Control Plasmid	20 µl (500 pg/µl)
C700210	SoluBL21™ Electrocompetent *E. coli.*	10 x 20 µl
	SOC Medium	6.0 ml
	pUC19 Positive Control Plasmid	20 µl (10 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.

Related Products	Catalog #
SoluLyse™ Bacterial Protein Extraction Reagent (Phosphate Buffer)	L100125 (125 ml); L100500 (500 ml)
SoluLyse™ Bacterial Protein Extraction Reagent (Tris Buffer)	L200125 (125 ml); L200500 (500 ml)
EZ-Spread ™ Beads, Single-Use Tubes	C400050 (50 tubes)
EZ-Spread ™ Beads, Dispenser Bottle	C400100 (1 bottle)
TurboCells® Competent *E. coli*	C300020 (20 x 50 µl)
TurboCells® BL21(DE3) Competent *E. coli*	C302020 (20 x 50 µl)
TurboCells® BL21(DE3) pLysS Competent *E. coli*	C303020 (20 x 50 µl)
SmartCells™ Competent *E. coli*	C101020 (20 x 50 µl)

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.

SoluBL21™ Strain: F^- *ompT hsd*S_B (r_{B^-} m_{B^-}) *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

† *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 ≥ 10^6 cfu/µg for chemically competent cells, and ≥ 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_2HPO_4	6 g
KH_2PO_4	3 g
NaCl	0.5 g
NH_4Cl	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**

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C. Chemical Transformation Protocol

1. Thaw one vial of chemically competent SoluBL21 cells on ice for a few minutes.
2. Transfer 50 µl 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 37ºC 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 37ºC.

D. Electroporation Protocol

9. Place 0.1 cm cuvette on ice for at least 5 minutes. Thaw cells on ice.
10. Add 1 µl of miniprep or ligation mix DNA directly to 20 µl 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 electroporator chamber.
14. Electroporate using 2.25 kV, 400 ohms, and 25 µF 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 OD_{600} = 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).

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