

SoluBL21™

Competent E. coli Kits

Cat. #	Contents	Quantity	Related Products	Catalog #
C700200	SoluBL21™ Chemically Competent E. coli	10 x 50P	SoluLys™ Bacterial Protein Extraction Reagent (Phosphate Buffer)	L100125 (125 ml); L100500 (500 ml)
	SOC Medium	6.0 ml	SoluLys™ Bacterial Protein Extraction Reagent (Tris Buffer)	L200125 (125 ml); L200500 (500 ml)
	pUC19 Positive Control Plasmid	20µl (500 pg/µl)	EZ-Spread™ Beads, Single-Use Tubes	C400050 (50 tubes)
C700210	SoluBL21™ Electrocompetent E. coli	10 x 20 µl	EZ-Spread™ Beads, Dispenser Bottle	C400100 (1 bottle)
	SOC Medium	6.0 ml	TurboCells® Competent E. coli	C300020 (20 x 50 µl)
	pUC19 Positive Control Plasmid	20µl (10 pg/µl)	TurboCells® BL21(DE3) Competent E. coli	C302020 (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.			
			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 this host bacterium an important tool for life science applications. However, a major obstacle faced by scientists using expression strains, such as BL21(DE3), is the high percentage of mammalian proteins that are expressed in insoluble form. Different approaches to dealing with protein insolubility in E. coli such as lowering expression temperature, changing promoters, purification tags, using alternative media, or plugging rework only in some cases and at a high cost in time, effort, and cost. With the SoluBL21 Competent E. coli, our scientists have used a novel directed evolution approach to create a significantly improved BL21(DE3) host strain. With this mutant strain, we will significantly improve their chances of obtaining partially or fully soluble proteins in the majority of expression experiments. In cases where partial solubility is achieved, users can obtain sufficient protein by simply increasing the size of their culture. With the new SoluBL21 strain, a major obstacle to soluble protein expression has been overcome for many mammalian proteins. This significant improvement should enable users to explore a wide range of applications more quickly and easily than in the past.

SoluBL21™ Strain	ompT hsdR (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

† The SoluBL21 strain contains uncharacterized mutations obtained through special selection criteria. These mutations are expected to improve the yield of soluble proteins in soluble form, fully or partially, in most tests conducted.

METHODS AND PROCEDURES

A. General Notes

The SoluBL21 transformation efficiency is 10^{10} cfu/µg for chemically competent cells, and 10^9 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 is optimized for use with M9 Minimal Media (M9). Prepare the M9 media as follows:

- 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

- 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.

- 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 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 electroporator 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 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 with OD
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 temperature 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 Protein Expression Kits (hereto "SoluBL21") end users a non-transferable, exclusive license to use the kits and/or their components for internal noncommercial research purposes only; in particular, this license does not include the right to use the kits and/or their components for the making or selling of pharmaceuticals or other commercial products. This license is granted by Gene Therapeutics, Inc. (GTS). Additionally:

1. A separate license is required for any commercial use of these materials for research purposes or product purposes by any commercial entity. Information about commercial licenses is obtained from: The Office of Intellectual Property and Research, Brookhaven National Laboratory, Bldg. 475, P.O. Box 5000, Upton, New York 11973-5000, telephone (631) 344-7134.
2. No materials that contain the cloned copy of T7 gene for T7 RNA polymerase, may be distributed further outside of your laboratory, unless the recipient receives a copy of this license and agrees to be bound by its terms. This limitation applies to strains SoluBL21, BL21 Gen-X, BL21(DE3), BL21(DE3)pLysS, and any derivatives you may make of them.

SoluBL21 and/or its components are not to be used for human diagnostic or included/used in any drug intended for human use. Caution should be exercised in handling the kit components by following appropriate research laboratory practices.

You may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to the terms of this license. The laws of the State of California shall govern the interpretation and enforcement of the terms of this License.