BL21 Gen-X™

Competent *E. coli* Expression Kit*

**Patent Pending*

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
C600500	BL21 Gen-X™ Chemically Competent *E. coli*	10 x 50 µl
	SOC Medium	3 ml
	pUC19 Positive Control Plasmid	20 µl (500 pg/µl)
	Gen-X™ Induction Enhancer, 10X	2 x 10 ml

Shipping	Shipped on Dry Ice
Storage	Store the BL21 Gen-X cells and the pUC 19 at -70°C. Store the Gen-X Induction Enhancer and the SOC Medium at 4 °C or room temperature. Stable for 6 months.

Related Products	Catalog #
Gen-X™ Induction Enhancer, 10X	C601500 (500 ml)
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 BL21 Gen-X™ *E. coli* is a new generation of BL21(DE3) that provides significantly higher levels of recombinant protein expression and significantly reduced levels of background proteins when compared to previous generations of BL21(DE3). The BL21 Gen-X was created by Genlantis scientists through careful cycles of mutagenesis, enrichment, and testing, resulting in a new and more powerful protein expression strain. By reducing the growth rate and optimizing other characteristics, Genlantis has created a BL21(DE3) strain that is qualitatively distinct from previous BL21(DE3) *E. coli*. The results are striking: the BL21 Gen-X strain, along with the powerful Gen-X™ Induction Enhancer, will express more of what you want, and less of what you don't want *in vivo*. Furthermore, Genlantis scientists have found that the BL21 Gen-X Strain is able to express proteins that are not expressed or detected at all using other popular BL21 cells or kits. With the BL21 Gen-X Competent *E. coli* Expression Kit, there is now a better way to express your recombinant proteins in bacteria. The resulting higher yields and lower contaminant protein amounts will result in easier downstream purification and processing, saving you significant time and money.

BL21 Gen-X™ 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 cloning efficiencies and representations of methylated DNA

† *The BL21 Gen-X strain contains uncharacterized mutations that result in increased foreground, reduced background, and a slower growth rate.*

METHODS AND PROCEDURES

A. General Notes

- The BL21 Gen-X transformation efficiency is approximately 10^5 cfu/µg. We recommend testing efficiency by using 2 µl of the pUC19 Positive Control Plasmid with 50 µl of cells. Plate transformation mix on LB agar with 100 µg/ml carbenicillin.
- Please note that the Gen-X Induction Enhancer™ has a slight yellow color at the 10X concentration.

B. Media Preparation

The BL21 Gen-X *E. coli* strain 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**

Genlantis
Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com

C. Transformation Protocol

1. Thaw one vial of BL21 Gen-X 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 BL21 Gen-X 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. Protein Expression

9. Inoculate a colony of the BL21 Gen-X into M9 minimal media with appropriate antibiotic.
10. Grow overnight at 37°C in a shaking incubator at 200 rpm.
11. Dilute cells into the same media until OD_{600} = 0.2

    NOTE: if cells are stationary, the dilution is approximately 1:20
12. Grow cells at 37°C until OD600 = 0.4. This will take approximately 90-120 minutes.
13. **OPTIONAL**: Add 10x Gen-X Induction Enhancer so that the final concentration is 1x.

    NOTE: The Induction Enhancer is not essential for protein expression, but including it may significantly improve your results. To purchase additional Gen-X Induction Enhancer™, use Catalog Number C610500 (500 ml, 10X).
14. Add IPTG to a final concentration of 1 mM.
15. Incubate cells overnight** at 37°C, in a shaking incubator at 200 rpm.

    NOTE: Some recombinant proteins, when over-expressed, can be toxic to the *E. coli* host. If the OD_{600} of the culture after overnight expression is lower than the OD_{600} at the start of induction, it is likely that the target protein is toxic. If desired, the recombinant protein can be purified from the cell supernatant. Alternatively, the induction time can be decreased to 6 hours (or shorter depending on visual inspection of the culture). Lowering the temperature will also delay host cell lysis but may reduce expression relative to 37ºC.
16. Spin down the cells and process as desired. For soluble protein extraction, we recommend the SoluLyse™ Protein Expression Reagents (See Related Products table above).

Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com