**Introduction:** The BL21 Gen-X™ E. coli is a next generation genetic variant of the standard BL21(DE3) strain. The BL21 Gen-X strain was created through careful cycles of mutagenesis, enrichment, and testing to provide a new and more powerful protein expression bacteria. These special E. coli cells have a slower growth rate and characteristics that lead to significantly higher recombinant protein expression, while exhibiting much lower endogenous or background bacterial proteins. Our results are clear, 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 *in vivo*. Furthermore, Genlantis Scientists have also discovered that the BL21 Gen-X Strain was able to express proteins that were not expressed or detected using other popular BL21 cells or kits available on the market today. With the BL21 Gen-X Competent E. coli Expression Kit, there is now a better way to express your genes in bacteria: higher yields and lower contaminant protein amounts will provide you with easier downstream purification and processing as well.

### BL21 Gen-X™ Strain:

- **F^-ompT hsdS (r-B- m-B-)**
  - DE3: Encodes T7 lysozyme 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 (B- 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 whenever needed or desired.

- 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:

- **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.

#### 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. Incubate the mixture at 37°C for 1 hour.
5. Plate on LB plates to select for the transformant.
6. Incubate overnight at 37°C.
7.挑取单克隆，进行纯化。
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. 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 OD$_{600}$ = 0.4. This will take approximately 90-120 minutes.
13. 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 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.
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).

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