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.

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:**
  - \(\text{Na}_2\text{HPO}_4\) 6 g
  - \(\text{KH}_2\text{PO}_4\) 3 g
  - NaCl 0.5 g
  - \(\text{NH}_4\text{Cl}\) 1 g
  - Water up to 800 ml

- **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 (rB- mB-)** Improved cloning efficiencies and representations of methylated DNA

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.

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):

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mM CaCl₂</td>
<td>1 ml</td>
</tr>
<tr>
<td>1 M MgSO₄</td>
<td>1 ml</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.3% final</td>
</tr>
<tr>
<td>Sterile Water</td>
<td>up to 1L final</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Contents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C600500</td>
<td>BL21 Gen-X™ Chemically Competent E. coli</td>
<td>10 x 50 µl</td>
</tr>
<tr>
<td></td>
<td>SOC Medium</td>
<td>3 ml</td>
</tr>
<tr>
<td></td>
<td>pUC19 Positive Control Plasmid</td>
<td>20 µl (10 pg/µl)</td>
</tr>
<tr>
<td></td>
<td>Gen-X Induction Enhancer™, 10X</td>
<td>2 x 10 ml</td>
</tr>
</tbody>
</table>

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.

BL21 Gen-X™ Strain: F- ompT hsdS (r-m-m) gal dcm (DE3)†

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

**LIMITED LICENSE:** The purchase price paid for the BL21 Gen-X™ Competent E. coli Expression Kit (hereafter "BL21 Gen-X") 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 without the written approval of Genlantis, a division of Gene Therapy Systems, Inc. (GTS). Additionally:

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