Lucigen®
Simplifying Genomics

E. cloni® EXPRESS Chemically Competent Cells

IMPORTANT!
-80 °C Storage Required
Immediately Upon Receipt

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support

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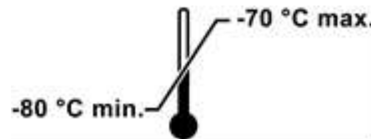
Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Components & Storage Conditions

Three strains of Lucigen's *E. cloni* EXPRESS Chemically Competent Cells are available: BL21(DE3), BL21(DE3)pLysS, and BL21(DE3)pLysE. All three strains of *E. cloni* EXPRESS Chemically Competent Cells have a transformation efficiency yield of $\geq$ 1 x 10^7 cfu/µg pUC19.

The cells are shipped on dry ice in one container, along with supercoiled control pUC19 DNA at 10 pg/µL and Expression Recovery Medium (lactose minus). *E. cloni* EXPRESS Chemically Competent Cells are available in 80 µL aliquots (DUOs), sufficient for 2 transformations per tube. Please refer to the table below for catalog numbers.

All *E. cloni* EXPRESS Competent Cells require storage at –80 °C.



E. cloni ® EXPRESS Chemically Competent Cells:

STRAIN	Efficiency (cfu/µg pUC19)	Transformations	Catalog #	Storage
E. cloni EXPRESS BL21(DE3) DUOs (Orange cap	$\geq$ 1 x 10^7	12 (6 x 80 µL) 24 (12 x 80 µL) 48 (24 x 80 µL)	60401-1 60401-2 60401-3	**-80 °C**
Expression Recovery Medium (lactose minus)	----	12 (1 x 12 mL) 24 (2 x 12 mL) 48 (4 x 12 mL)	----	**-20 to -80 °C**
Supercoiled pUC19 DNA (10 pg/µL)	----	(1 x 20 µL)	----	**-20 to -80 °C**

* Additional Expression Recovery Medium (lactose minus) can be ordered separately as Catalog # 80030-1 (8 x 12 mL)

E. cloni EXPRESS Chemically Competent Cells

E. cloni EXPRESS BL21(DE3) Chemically Competent Cells is an *E. coli* strain- ideal for routine protein expression applications. NOTE: for expressing toxic proteins, we recommend Lucigen's OverExpress™ C41(DE3) and C43(DE3) Competent Cells.

BL21(DE3)is a lysogen- of λDE3. This strain- carries a chromosomal copy of the T7 RNA Polymerase gene under the control of the *lac*UV5 promoter. This strain- is suitable for production of protein from target genes cloned into T7 driven expression vectors. E*. cloni* Express BL21(DE3is also deficient in the *lon and ompT* proteases.

Genotype

***E. cloni* EXPRESS BL21(DE3)**
F^{-} *ompT hsdS*$_B$ *(*r_B^- m_B^-*) gal dcm* (DE3)

As a control for transformation, *E. cloni* EXPRESS Chemically Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/µL. Use 1 µL for transformation.

Preparation for Transformation

E. cloni EXPRESS Chemically Competent Cells are provided in aliquots of 80 µL sufficient for two transformation reactions of 40 µL each.

Transformation is performed by heat shock at 42 °C, followed by incubation on ice.

To ensure successful transformation results, the following precautions should be taken:

- **For best results, Lucigen CloneSmart® ligation reactions must be heat inactivated at 70 °C for 15 minutes before transformation. Alternatively, the reactions may be purified. For other ligation** reactions, follow the manufacturer's recommendations.
- All tubes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Expression Recovery Medium to resuspend the cells after transformation.
- Perform the heat shock in a **15-mL disposable polypropylene culture tube** (17 x 100 mm). The use of other types of tubes may dramatically reduce the transformation efficiency

Transformation Protocol

1. Prepare nutrient agar (LB-Lennox) plates with antibiotic for selection. Remove Recovery Medium from the freezer and bring to room temperature.
2. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).
3. Remove E. cloni EXPRESS cells from the -80 °C freezer and thaw completely on wet ice (5-15 minutes).
4. Add 40 µL of E. cloni EXPRESS cells to the chilled culture tube.
5. Add 1 µL of DNA to the 40 µL of cells. Stir briefly with a pipet tip; do not pipet up and down to mix, which can introduce air bubbles and warm the cells. For the pUC19 control, add 1 µL (10 pg) of DNA to another culture tube containing 40 µL of cells. Stir briefly.

   Note: Lucigen CloneSmart® ligation reactions must be heat-inactivated or purified. For other ligation reactions, follow the manufacturer's recommendations.
6. Incubate the cell/DNA mixture on ice for 30 minutes.
7. Heat shock cells by placing the culture tubes in a 42 °C water bath for 45 seconds. ***Performing the heat shock in the 1.7 mL tube in which the cells are provided will significantly reduce the transformation efficiency.***
8. Return the tubes to ice for 2 minutes.
9. Add 960 µL of room temperature Expression Recovery Medium to the cells in the culture tube.
10. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37 °C.
11. Plate up to 200 µL of the transformation on LB-Lennox agar plates containing the appropriate antibiotic. The plating volume may need to be optimized depending on your DNA.
    For the pUC19 control, plate 200 µL of the transformation on LB-Lennox agar plates containing 100 µg/mL carbenicillin or ampicillin.

12. Incubate the plates overnight at 37 °C.
13. Transformed clones can be further grown in LB or any other lactose-minus medium.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as the number of transformants (in cfu) per µg of plasmid DNA.

$$\frac{\text{\# cfu}}{\text{pg pUC 19 DNA}} \times \frac{10^6 \text{ pg}}{\text{ug}} \times \frac{\text{volume of transformants}}{\text{X µL plated}} \times \text{dilution factor} = \text{cfu/µg}$$

For example, if 10 pg pUC yields 10 colonies when 100 µL of a 1 mL transformation is plated, then:

$$\frac{\text{10 cfu}}{\text{10 pg pUC19}} \times \frac{1\text{x}10^6 \text{ pg}}{\text{µg}} \times \frac{\text{1000 µL}}{\text{100 µL}} = 1.0 \times 10^7 \text{ cfu/µg pUC19}$$

Sample Induction Protocol

1. Inoculate a single colony from a freshly streaked plate into 5 mL of LB medium containing the appropriate antibiotic for the plasmid and host strain.
2. Incubate with shaking at 37 °C overnight. To minimize the expression of the target protein prior to induction, add glucose to the growth medium to a final concentration of 0.2% (w/v).
3. Inoculate 50 mL of LB medium containing the appropriate antibiotic with 0.5 mL of the overnight culture prepared in step 2.
4. Incubate with shaking at 37 °C until the OD_{600} reaches 0.6 - 0.8.
5. Add IPTG to a final concentration of 1 mM. To determine the optimal concentration of IPTG for maximum expression of the target protein test a range of IPTG concentrations from 0.25 – 2 mM.
6. Incubate at 37 °C for 3-4 hours. The optimal time for induction of the target protein may vary from 2-16 hours.
7. Place the culture on ice for 10 minutes. Harvest cells by centrifugation at 5,000 x g for 10 minutes at 4 °C.
8. Remove the supernatant and store the cell pellet at -20 °C (storage at lower temperatures is also acceptable).

Media Recipes

LB Lennox Agar Plates

Per liter:
10 g tryptone
5 g yeast extract
5 g NaCl
15 g agar

Medium for Growth of Transformants
LB Miller

Per liter:
10 g tryptone
5 g yeast extract
10 g NaCl

Add all components to deionized water. **Adjust pH to 7.0 with NaOH.** Autoclave and cool to 55 °C.

TB

Per liter: 11.8 g tryptone
23.6 g yeast extract
9.4 g dipotassium hydrogen phosphate (anhydrous)
2.2 g potassium dihydrogen phosphate (anhydrous)
0.4% glycerol
Add all components to deionized water**.** Autoclave and cool to 55 °C.

IPTG

Prepare a 1 M solution of IPTG **(Isopropyl-β-D-thiogalactoside; Isopropyl-β-D-thiogalactopyranoside)** by dissolving 2.38 g of IPTG in water and adjust the final volume to 10 mL. Filter sterilize before use.

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