Lucigen®
Simplifying Genomics

E. cloni® EXPRESS Chemically Competent Cells

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Table of Contents

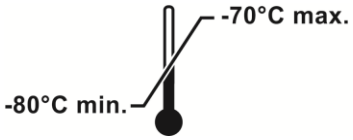
Components & Storage Conditions....2
E. cloni EXPRESS Chemically Competent Cells....3
Preparation for Transformation....3
Transformation Protocol....4
Media Recipes....5
Related Products....5

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 $\geq 1 \times 10^7$ 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 tubes)	$\geq 1 \times 10^7$	12 (6 x 80 µl) 24 (12 x 80 µl) 48 (24 x 80 µl)	60401-1 60401-2 60401-3	**-80°C**
E. cloni EXPRESS BL21(DE3)pLysS DUOs (Pink tubes)	$\geq 1 \times 10^7$	12 (6 x 80 µl) 24 (12 x 80 µl) 48 (24 x 80 µl)	60413-1 60413-2 60413-3	**-80°C**
E. cloni EXPRESS BL21(DE3)pLysE DUOs (Purple tubes)	$\geq 1 \times 10^7$	12 (6 x 80 µl) 24 (12 x 80 µl) 48 (24 x 80 µl)	60425-1 60425-2 60425-3	**-80°C**
E. cloni EXPRESS BL21(DE3) ChemComboPack (4 rxn of each strain)		12 (6 x 80 µl)	60430-1	**-80°C**
Expression Recovery Medium (lactose minus)		12 (1 x 12 mls) 24 (2 x 12 mls) 48 (4 x 12 mls)	----	**-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), BL21(DE3)pLysS, and BL21(DE3)pLysE Chemically Competent Cells are *E. coli* strains that are 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), BL21(DE3)pLysS, and BL21(DE3)pLysE are lysogens of λDE3. These strains carry a chromosomal copy of the T7 RNA Polymerase gene under the control of the *lac*UV5 promoter. These strains are suitable for production of protein from target genes cloned into T7 driven expression vectors. E*. cloni* Express BL21(DE3), BL21(DE3)pLysS, and BL21(DE3)pLysE are also deficient in the *lon and ompT* proteases.

E. cloni EXPRESS BL21(DE3)pLysS, and BL21(DE3)pLysE have the same chromosomal genotype as BL21(DE3), These strains also carry a chloramphenicol resistant plasmid that encode T7 lysozyme which is a natural inhibitor of T7 RNA Polymerase. Cells containing pLysS produce a small amount of T7 Lysoyme, whereas the pLysE strain produces considerably more T7 Lysozyme. These strains are used to suppress basal expression of T7 RNA polymerase prior to induction, thus stabilizing recombinants encoding potentially deleterious proteins.

Genotypes

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

***E. cloni* EXPRESS BL21(DE3)pLysS**
F⁻ *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3) pLysS (Cm^R)

***E. cloni* EXPRESS BL21(DE3)pLysE**
F⁻ *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3) pLysE (Cm^R)

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, respectively, 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 must be taken:

- For best results, the ligation reaction must be purified or heat killed at 70ºC for 15 minutes before transformation.
- All microcentrifuge 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.

Transformation Protocol

1. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).
2. Remove *E. cloni* EXPRESS cells from the -80°C freezer and thaw completely on wet ice (10-20 minutes).
3. Add 40 µl of *E. cloni* EXPRESS cells to the chilled culture tube.
4. Add 1 µl of ligation reaction or DNA sample to the 40 µl of cells on ice. (Failure to purify or heat-inactivate the ligation reaction may prevent transformation.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
5. Incubate on ice for 30 minutes.
6. Heat shock cells by placing them in a 42^{o}C water bath for 45 seconds.
7. Return the cells to ice for 2 minutes.
8. Add 960 µl of room temperature Expression Recovery Medium to the cells in the culture tube.
9. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37^{o}C.
10. Plate up to 100 µl of transformed cells on LB agar plates containing the appropriate antibiotic.
11. Incubate the plates overnight at 37°C.
12. Transformed clones can be further grown in LB or any other lactose minus medium.

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 (Prepare a 1 M solution of IPTG by dissolving 2.38 g of IPTG in water and adjust the final volume to 10 ml. Filter sterilize before use). 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 Culture Medium for Growth of Transformants

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

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

LB Agar Plates

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

Add deionized water to 1 liter. Adjust pH to 7.0 with NaOH. Autoclave. Cool to 55°C and add the appropriate filter-sterilized antibiotic (e.g., 30-50 mg kanamycin for kanamycin-resistant transformants; 100 mg ampicillin or carbenicillin for ampicillin-resistant transformants).

For blue/white screening, add 3 ml 100mM IPTG and 10 ml 2% X-gal to the molten agar at 55°C before pouring.

Pour approximately 25 ml per petri plate.

Related Lucigen Products

- Expresso™ T7 Cloning & Expression System
- OverExpress™ Competent Cells
- CloneSmart® Blunt Cloning Kit
- DNATerminator® End Repair Kit
- PCRTerminator® End Repair Kit
- UltraClone™ DNA Ligation & Transformation Kit
- CloneDirect™ Rapid Ligation Kit
- PCR-SMART™ Cloning Kit
- ClonePlex® Library Construction Kit
- pEZSeq™ Blunt Cloning Kit
- cSMART™ cDNA Cloning Kit
- *E. cloni*® 10G Competent Cells

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