Acella™ pLysS Chemically Competent Cells

Product	Cat. No.	Transformations
Acella™ pLysS Chemically Competent Cells, Tubes	10773	12

Description

Acella™ pLysS Chemically Competent Cells are *ΔendA ΔrecA* derivatives of BL21 (DE3) cells that have been manufactured using proprietary technology to make the cells highly efficient for DNA uptake, thus ultra competent. To utilize the cells at their highest efficiency, a recommended transformation protocol is included with each kit.

The complete deletions of the *endA* and *recA* genes eliminate plasmid recombination and provide excellent yield and quality plasmid DNA, making this strain ideal to combine direct cloning and protein expression. By cloning directly in the Acella™ pLysS strain, you save at least two days of work normally spent on subcloning and you eliminate the need for additional highly efficient competent cells for cloning procedures.

Cloning and expression steps can be combined by directly transforming the Acella™ pLysS Chemically Competent Cells with the ligation products using the enclosed protocol. Transformed cells should be plated in selective medium and incubated at 37°C overnight. Since Acella™ pLysS Chemically Competent Cells are a fast growing strain, miniprep cultures can be grown for 3-4 hours and plasmids can be analyzed the day after the transformation, saving oneextra day. Positive clones can then be grown directly for protein expression.

Like all BL21 cells, Acella™ pLysS Competent Cells lack the Lon and OmpT proteases, promoting stability of recombinant proteins. Acella™ pLysS Competent Cells carry a copy of the T7 RNA polymerase and are ideal for expression of T7 promoter-driven constructs. Additionally, for toxic proteins and tighter control of basal expression, Acella™ pLysS Competent Cells express T7 lysozyme, a T7 RNA polymerase inhibitor, reducing background levels of polymerase activity.

Acella™ pLysS Chemically Competent Cells are available in single use tubes that provide a simple and reliable method for high-efficiency, single use transformation. All kits include a test plasmid for quality control purposes. Cells are pre-dispensed in 50µl aliquots.

Full processing time (including recovery) is about 1 hour 20 minutes to ensure the highest level of transformation. Edge BioSystems guarantees transformation efficiencies of Acella™ pLysS Chemically Competent Cells exceeding $2x10^8$ cfu/µg pUC19.

Genotype

Acella™ pLysS Chemically Competent Cells: F^- *ompT* *hsd*$S_B(r_B^-m_B^-)$ *gal dcm* (DE3) pLysS (Cam^R) *ΔendA ΔrecA*

Kit Components	10773
Acella™ pLysS Chemically Competent Cells	12 tubes
pUC19 Supercoiled DNA, 100ng/ml	1 tube

Quality Control

Each lot is tested to assure high transformation efficiency using 10pg pUC19 supercoiled DNA and the recommended protocol. Transformation efficiency will be ≥ $2x10^8$ cfu/µg pUC19, under these conditions. The presence of the pLysS plasmid is confirmed by plating the cells in medium containing chloramphenicol.

Equipment and Materials Not Provided

1. A 42°C water bath.
2. 14ml round-bottom culture tubes (one per tube of Acella™ pLysS Chemically Competent Cells).
3. An orbital shaker capable of 37ºC and 320 rpm.
4. SOC medium for recovery: 20g/l tryptone, 5g/l yeast extract, 10mM NaCl, 2.5mM KCl, 10mM $MgCl_2$, 10mM $MgSO_4$, 20mM glucose ($MgCl_2$, $MgSO_4$ and glucose should be added after autoclaving).
5. LB-agar plates or liquid media containing the appropriate antibiotic and chloramphenicol (final chloramphenicol concentration of 35µg/ml in ethanol) for selection of the pLysS plasmid.

Storage Conditions

Acella™ pLysS Chemically Competent Cells should be stored in a -80ºC freezer. Please note that competent cells are very sensitive to cycles of freezing and thawing and should not be exposed to temperature variations.

Recommended Protocol for Tubes

1. Immediately after taking the tubes from the -80°C freezer, place them on ice for approximately 5 minutes to thaw.

2. Pipette the DNA to be transformed to the bottom of the tube and mix by pipetting 50 µl of air to the bottom of the tube. Control transformation: Dilute pUC19 supercoiled DNA 1:10 with dH_2O, then add 1 µl of the diluted pUC19 supercoiled DNA to one of the tubes. Discard the remaining diluted pUC19 supercoiled DNA after use.

   ***Note**:* **Do not mix by pipetting up and down since that will lower the transformation efficiency.**

3. Incubate the tubes on ice for 10 minutes.

4. Transfer the tubes to a 42°C water bath, incubate for 40 seconds and transfer back to ice.

5. Incubate the tubes for 2 minutes on ice.

6. Transfer the cells into a 14 ml round-bottom culture tube filled with 1 ml of pre-warmed SOC medium and then shake at 300 rpm at 37°C for 1 hour.

7. Plate cells on pre-warmed LB-agar selective plates or incubate into selective liquid medium. For the control transformation with pUC19 supercoiled DNA, plate 10 µl on LB-ampicillin agar plates and expect >20 colonies (>2 x 10^8 cfu/µg pUC19).

Additional Notes

a. Transformation efficiencies for ligation mixtures will be 10-100 fold lower than for pUC19 supercoiled DNA.

b. Calculation of transformation efficiency

[(cfu on control plate) / (pg of supercoiled pUC19)] x (10^6 pg / µg) x (final dilution) = cfu / µg DNA

Note: cfu = colony forming units

Special Note

Acella™ pLysS Chemically Competent Cells are based on the T7 expression system. This technology was developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy. Consequently, U.S. patents assigned to Brookhaven Science Associates (BSA) protect this technology. These materials are to be used by noncommercial entities for research purposes only. Commercial entities require a license from BSA. You may refuse these cells by returning the enclosed materials unused. To obtain information about licensing, please contact the Office of Intellectual Property and Partnerships, Brookhaven National Laboratory, Building 475D, Upton, NY 11973 (telephone: 631-344-7134 or fax: 631-344-3729).