Ultra BL21 (DE3) pLysS Competent Cells

Product	Cat. No.	Transformations
Ultra BL21 (DE3) pLysS Competent Cells, Tubes	43066	12

Description

Ultra BL21 (DE3) pLysS Competent Cells are chemically competent and have been manufactured using proprietary technology making 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.

Ultra BL21 (DE3) pLysS Competent Cells are available in single use tubes that provide a simple and reliable method for high-efficiency, single use transformation of host cells. All cells include a test plasmid for quality control purposes. Cells are pre-dispensed in 50µl aliquots (tubes). Full processing time for 1 tube (including recovery) is about 1 hour and 20 minutes to ensure the highest level of transformation. Edge BioSystems guarantees transformation efficiencies that exceed $2x10^8$ cfu/µg pUC19.

Genotype: F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3) pLysS (Cam^R).

Kit Components 43066

Ultra BL21 (DE3) pLysS 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 exceed $2x10^8$ 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. SOC medium for recovery: 20g/l tryptone, 5g/l yeast extract, 10mM NaCl, 10mM KCl, 10mM $MgCl_2$, 10mM $MgSO_4$, 20mM glucose ($MgCl_2$, $MgSO_4$ and glucose should be added after autoclaving).
2. An orbital shaker.
3. A 42°C water bath.
4. 14ml round bottom culture tubes (1 tube per single use aliquot).
5. LB-agar plates or liquid medium containing the appropriate antibiotic. Chloramphenicol (35µg/ml in ethanol) should be added to plates or liquid medium for selection of the pLysS plasmid.

Storage Conditions

Ultra BL21 (DE3) pLysS 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 in ice and wait approximately 5 minutes until they thaw.
2. Pipette the DNA to be transformed to the bottom of the tube and mix by pipetting 50µl of air to the bottom of the tube. Control transformation: Dilute pUC19 supercoiled DNA 1:10 with sterile diH_2O, then add 1µl of the diluted pUC19 supercoiled DNA to one of the tubes. Discard 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 in 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 in ice.
6. Transfer the cells into a 14ml round bottom culture tube filled with 1ml 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 containing chloramphenicol or inoculate into selective liquid medium containing chloramphenicol. For the control transformation with pUC19 supercoiled DNA, plate 10µl on LB-ampicillin agar plates and expect >20 colonies (>2 x 10^8 cfu/µg pUC19).

Special Note

Ultra BL21 (DE3) pLysS 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.

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