BL21(DE3) Chemi-Competent Expression Cells 156-3003

10 x 0.05 ml

For research use only
Store at –70°C

Storage and Stability

Store the BL21 (DE3) competent cells at –70°C in a constant temperature freezer. When stored under these conditions, the cells are stable for 6 months after date of receipt.

Kit Contents

10 x 50 µl BL21 (DE3) chemically competent cells

1 x 10 µl pUC19 control plasmid DNA (10 ng/µl) in 10 mM Tris-HCl pH 8.0, 1 mM EDTA

1 x 10 ml SOC medium*

* (2% Tryptone, 0.5% yeast extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 5 mM $MgCl_2$, 5 mM $MgSO_4$)

Description

BL21 (DE3) competent cells are chemically competent *E. coli* cells used for high-level protein expression with T7 RNA polymerase-based expression systems. The strain is a derivative of *E. coli* B, which is deficient in the lon protease as well as the ompT outer membrane protease, facilitating the isolation of intact recombinant proteins. The host is a lysogen of λDE3 and contains the T7 RNA polymerase gene, under the control of the *lac*UV5 promoter, integrated into the chromosome. IPTG is used to induce the expression of recombinant proteins cloned into vectors downstream of a T7 RNA promoter and transformed into the BL21 (DE3) cells.

BL21 (DE3) competent cells are packaged in single-use volumes for convenience and maintenance of efficiency. The recommended transformation protocol will yield 10 x 50 µl transformations.

Genotype

E. coli B F^- *dcm ompT hsdS*(r_B^-, m_B^-) *gal λ(DE3)*

Quality Control

Each batch of BL21 (DE3) competent cells is performance tested to ensure yields of ≥1 x 10^7 CFU/µg DNA using nonsaturating amounts of pUC19 control plasmid.

Protocol Notes

1. Thaw cells on wet ice. For best results, use within 15 min of thawing. Refreezing thawed competent cells will result in decreased transformation efficiencies.
2. Mix cells by gently tapping (flicking) the reaction tube. Do not pipet the cells or vortex the tube.
3. For highest transformation efficiencies, follow the recommended time for the duration of the 42°C heat pulse.

Transformation Protocol

1. Thaw on ice one tube of 50 µl competent cells for each transformation. Gently mix the cells by tapping the tube. Once thawed, cells should be used promptly.
2. For determining the transformation efficiency, add 1 µl (10 ng) of pUC19 control DNA to one tube of competent cells. Gently mix by tapping the tube.

3. For experimental DNA, add 1 to 50 ng DNA (in ≤ 5 µl) to one tube of cells. Gently mix by tapping the tube. For best results, the experimental DNA should be free of protein, detergents, organic solvents, and salts.
4. Incubate cell/DNA mix on ice for 30 min.
5. Heat shock the cells by placing tubes in a 42°C water bath for 30 sec.
6. Return the tubes to ice for 2 min.
7. Add 250 µl room temperature SOC medium to each transformation reaction.
8. Secure the tubes in a microcentrifuge tube rack with tape.
9. Place the rack on its side in a shaking incubator. Incubate at 37°C for 1 hr while shaking at 225–250 rpm.
10. Spread up to 200 µl of the transformed cells on LB agar plates containing the appropriate antibiotic (for example 100 µg/ml ampicillin for the pUC19 control plasmid and for Profinity eXact™ tag plasmids). Note: Spreading the liquid until the surface of the plate is dry can significantly reduce the number of transformants.
11. Incubate plates 12–16 hr at 37°C.
12. Transformation efficiency (CFU/µg) may be calculated as follows:

$$\frac{\text{CFU on plate}}{\text{ng pUC19 added}} \times \frac{1 \times 10^3 \text{ ng}}{\mu\text{g}} \times \frac{\text{Transformation volume}}{\text{volume plated}} \times \text{dilution factor} = \text{CFU}/\mu\text{g}$$

Example:

If a transformation experiment using 1 ng of pUC19 DNA results in 150 colonies after plating 100 µl of a 1:50 dilution, transformation efficiency is:

$$\frac{150 \text{ colonies}}{1 \text{ ng}} \times \frac{1 \times 10^3 \text{ ng}}{\mu\text{g}} \times \frac{0.25 \text{ ml}}{0.1 \text{ ml}} \times 50 = 1.88 \times 10^7 \text{ CFU}/\mu\text{g}$$

Related Products

Catalog#	Description
156-3001	**Profinity eXact pPAL RIC-Ready Expression Vector Kit**
156-3002	**Profinity eXact pPAL Supercoiled Expression Vector Kit**
156-3008	**Profinity eXact Expression and Purification Starter Kit**
156-3006	**Profinity eXact Mini Spin Purification Starter Kit**, 10 pk
156-3005	**Profinity eXact Purification Resin**, 10 ml
732-4646	**Bio-Scale Mini™ Profinity eXact cartridges**, 2 x 1 ml
732-4648	**Bio-Scale Mini Profinity eXact cartridge**, 1 x 5 ml

Legal Information

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