BL21(DE3) Competent Cells

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Thermo Scientific™ BL21(DE3) Competent Cells are suitable for the expression of nontoxic heterologous genes. The strain contains the DE3 lysogen that carries the gene for T7 RNA polymerase under control of a *lac*UV5 promoter, allowing expression of the T7 RNA polymerase to be induced with IPTG. BL21(DE3) is a B/r strain and does not contain the *lon* protease. It is also deficient in the outer membrane protease, OmpT. The lack of these two key proteases reduces degradation of heterologous proteins expressed in the cells.

Genotype

F^- *omp*T *hsd*SB (rB-mB-) *gal dcm* (DE3)

Contents and storage

Contents	Amount	Storage
BL21(DE3) Competent Cells	10 × 100 µL	–80°C (Do not store in liquid nitrogen)
pUC19 DNA (10 pg/µL)	50 µL	–80°C
S.O.C. Medium	6 mL	4°C or room temperature

Guidelines for tranforming cells

- Only use BL21(DE3) competent cells for **protein expression**. To propagate or maintain an expression plasmid, use competent cells that does not carry the gene for T7 RNA polymerase (i.e., TOP10, DH5α).
- For best results, thaw each vial of cells only once. Subsequent freeze-thaw cycles significantly lower transformation efficiency.
- Use S.O.C. Medium for the cell recovery procedures after heat shock. Transformation efficiency is reduced if other types of media are used.
- Experimental DNA should be free of phenol, ethanol, protein, and detergents to obtain maximum transformation efficiency.
- To determine the transformation efficiency of the cells, perform a control reaction using 10 pg (1 µL) of the pUC19 DNA stock solution. Spread 30 µL of the pUC19 DNA control reaction on a LB plate containing 100 µg/mL of ampicillin.

Transform competent cells

1. Thaw competent cells on wet ice. Place the required number of 1.5-mL polypropylene microcentrifuge tubes on wet ice.
2. Gently mix the cells, then make 50 µL aliquots of competent cells in the chilled 1.5-mL microcentrifuge tubes.
3. Add 1–5 µL of sample DNA directly into a tube of competent cells. Mix well by gently flicking tube several times.
4. Incubate the cells on ice for 30 minutes.
5. Heat-shock the cells for exactly 30 seconds in a 42°C water bath.

   Do not mix or shake the tube.
6. Incubate the cells on ice for 2 minutes.
7. Add 250 µL of room-temperature S.O.C. Medium.
8. Place the tube on its side in a shaking incubator. Use tape to secure the tube in place.
9. Shake the tube at 225 rpm for 1 hour at 37°C.
10. Spread at least two different volumes (20–200 µL) of cells from each transformation reaction on separate LB plates containing the appropriate selective antibiotic. Label the plates with the plating volume so that the amount providing the best colony density can be identified.
11. Invert the plates and incubate overnight at 37°C.

Calculate transformation efficiency

Transformation efficiency can be 10- to 100-fold lower for transformation of a ligation reaction mixture than for transformation of an intact plasmid such as pUC19 DNA.

Calculate the transformation efficiency (CFU/µg) as follows:

$$\frac{\text{CFU in plate}}{\text{pg of DNA used in transformation}} \times \frac{1 \times 10^6\ \text{pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

For example, if 10 pg of pUC19 DNA yields 50 colonies when 30 µL is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{50\ \text{CFU}}{10\ \text{pg}} \times \frac{1 \times 10^6\ \text{pg}}{\mu\text{g}} \times \frac{300\ \mu\text{L}}{30\ \mu\text{L plated}} \times 1 = 5 \times 10^7$$

For Research Use Only. Not for use in diagnostic procedures.

Guidelines for protein expression

- Choose 3–4 transformants when characterizing clones for protein expression because clones may exhibit differences in expression of the heterologous genes.
- IPTG is required to induce expression of the T7 RNA polymerase from the *lac*UV5 promoter.
- For best results, use BL21(DE3) competent cells to express **non-toxic** heterologous genes. Because of the extremely high activity of T7 RNA polymerase, some basal level expression of the gene of interest may occur in uninduced cells. This creates problems in cases where the gene of interest is toxic to bacterial cells. In such cases, uninduced expression of a toxic gene can lead to selection of cells that express the low levels of the toxic protein. These cells are often unable to express high levels of the gene of interest upon IPTG induction.

Perform protein expression

1. Pick 3–4 transformants and inoculate each one in 5 mL of LB medium containing the appropriate selective antibiotic for your expression plasmid.
2. Grow the cultures overnight at 37°C with shaking until they reach saturation (OD_{600} ≥2).
3. Use the overnight cultures to inoculate fresh LB medium containing antibiotic to an OD_{600} of 0.05–0.1 (~1:50 dilution of the overnight culture). Use a volume appropriate for taking time points, if desired.

   Note: This dilution allows the cells to quickly return to logarithmic growth and reach the appropriate cell density.
4. Use the remainder of each overnight culture to create glycerol stocks. Once you have identified the clone that best expresses your protein, you can use the glycerol stock to perform additional expression experiments.
5. Incubate the cultures for 2–3 hours until they reach mid-log phase (OD_{600} ~0.4).
6. Add IPTG to a final concentration of 0.5 mM to induce the cultures, then incubate for an additional 2–3 hours.

   You can take time points to analyze for optimal expression of your protein.
7. Analyze clones by western blot or enzymatic assay to determine which clone best expresses your protein of interest.

   Note: Use the glycerol stock created from this clone for expression experiments. If you find that expression levels in subsequent inductions decrease, or you find that you lose your plasmid, your protein may be toxic to *E. coli*.

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