PRODUCT INFORMATION

Product Name : Electrocompetent Cell BL21(DE3)
Code No. : DS258
Size : 50 µl × 5
Competency : > 1×10^9 cfu/µg (50 µl cell, pBR322)
Supplied product : SOC medium, 1 ml × 5 *This product is for research use only*

Description :

Electrocompetent Cell BL21(DE3) is designed for high efficiency transformation by electroporation and is suitable for DNA library construction and all kinds of cloning procedures. *E coli* strain BL21(DE3) is a widely used strain for bacterial protein expression. The BL21(DE3) strain contains the λDE3 lysogen, which expresses T7 RNA polymerase under the control of the *lac*UV5 promoter[1)]. Upon addition of IPTG, T7 RNA polymerase is expressed and it induces a high-level protein expression from T7 promoter driven expression vectors (e.g., pET).

Genotype of *E coli* strain BL21(DE3) : F^- *ompT hsdS*(r_B^- m_B^-) *gal dcm* λ(DE3)
(λ(DE3): *lac*I, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

Quality control :

Transformation by electroporation was carried out according to the method described in this Product Information using supercoiled pBR322 plasmid. Transformants were plated on LB plates containing 50 µg/ml ampicillin. The efficiency was confirmed to be greater than 1×10^9 cfu/µg with 50 µl of the cells.

Storage condition :

Stable at -80℃ with little or no loss in transformation efficiency for 12 months from the date of receipt. Upon receipt, store the Electrocompetent Cell DH5α in a freezer at -80°C directly from a dry ice shipping box and store SOC medium at room temperature or at -80°C.

Handling of competent cells :

• After thawing electrocompetent cells on ice, cells tend to lose transformation efficiency gradually. Transformation should be started immediately following thawing cells on ice.
• Electroporation is temperature-dependant. Electrocompetent cells should be maintained at 0-4°C before pulsing with electroporation apparatus.
• Use of refrozen electrocompetent cells is not recommended.

Composition of SOC medium supplied :

20 g/L	tryptone
5 g/L	yeast extract
0.5 g/L	NaCl
0.186 g/L	KCl
2.4 g/L	$MgSO_4$, anhydrous
4 g/L	glucose

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Transformation (electroporation) procedure :

● Materials to be supplied by user

- Electroporation apparatus
- Sterile electroporation cuvettes (0.1 cm gap)
- LB plates with antibiotic
- Sterilized pasteur pipettes
- Sterile 14 ml polypropylene culture tubes
- 37°C shaker
- Sterile spreaders
- 37°C incubator

● Transformation

Before start:

- Chill sterile electroporation cuvettes (0.1 cm gap) on ice.

  * Electrocompetent cells should be maintained at 0-4°C before pulsing.
- Place 14 ml sterile polypropylene culture tubes containing 950 µl of SOC medium (at room temperature) for each electroporation near the electroporation apparatus.

  * It is important to add SOC medium to electrocompetent cells immediately after pulsing.
- Set up the electroporation apparatus according to the manufacturer's instructions.

1. Thaw the electrocompetent cells on ice and add DNA solution*(1-3 µl) to the cells then flick gently.

   * DNA solution*: Highly purified DNA is recommended. DNA should be dissolved in pure water or 1×TE. For ligation mixture, see "To use ligation mixture for electroporation" below.
2. Quickly transfer the mixture of cells and DNA to a chilled electroporation cuvette, and tap the mixture to the bottom of the cuvette.

   * Electroporation should be performed immediately after mixing electrocompetent cells and DNA.
3. Place the cuvette into the electroporation apparatus, then pulse.
4. Remove the cuvette from the electroporation apparatus and immediately add the 950 µl of SOC medium to the cuvette with a sterile pasteur pipette to suspend cells.
5. Transfer the cell suspension to the sterile 14 ml polypropylene culture tube. Culture the cell at 37°C for 1 hr in a shaker.
6. Spread aliquot of the cell to an LB agar plate containing appropriate antibiotic.
7. Incubate the plate at 37°C overnight.

● To use ligation mixture for electroporation

Ligation mixture contains salts and impurities that interfere with electroporation. To use ligation mixture for electroporation, DNA should be purified by phenol/chloroform extraction following ethanol precipitation or with a spin column. The phenol/chloroform extraction following ethanol precipitation gives successive results for ligation mixture prepared with DNA Ligation Kit ver. 2 (DS110).

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● Notes for transformation

1. As T7 expression method is a high-level protein expression system, some basal level expression of the target protein will occur in uninduced cells. This is likely to be problematic in cases in which the target protein is toxic to *E. coli.* cells. In this case, it may be necessary to decrease the basal level expression as follows:
   1) Use a low-copy, T7 driven expression vector.
   2) Use liquid medium and agar plates supplemented with glucose (0.5 -1 %).
      Glucose is known to decrease a basal expression from *lac*UV5 promoter[2)].
2. Expression may vary among transformants. If large and small colonies are observed in the same plate, the expressed protein may affect the growth of the *E. coli* cells.
3. If the expressed protein is toxic to *E. coli* cells, transformants may not be obtained.

Protein expression procedure :

The following protocol is a general guide for the protein expression by use of T7 promoter driven expression vectors and BL21(DE3) cells.

Before starting:

1. Construct T7-promoter expression plasmid harboring a gene of interest using non-expression hosts.
2. Transform Electrocompetent Cell BL21(DE3) with the expression plasmid.

Expression:

1. Following electroporation, pick a colony and inoculate it into 3 ml of LB medium containing the appropriate antibiotic with shaking at 37°C, overnight.
2. The next morning, transfer 0.5 ml of the overnight culture to a new 10 ml of LB medium containing the appropriate antibiotic to select the expression plasmid. Grow the culture with shaking at 37°C until the OD_{600} reaches 0.5 (approximately 2 hrs but this depends on the expression plasmids).
3. When the OD_{600} reaches 0.5, add IPTG to a final concentration of 1 mM to the rest of the culture and grow the culture with shaking at 37°C for 3 hours. The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.
4. After the induction, harvest the cells.

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Reference:

1) Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.

2) Pan, S. and Malcom, B.A., *BioTechniques* 29 (2000), 1234–1238.

General reference in this Product Information

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

● Purchaser Notification

This product is manufactured based on the T7 expression system which is the subject of US patent applications assigned to Brookhaven Science Associates, LLC (BSA). The product must be used only outside the United Stated and its territories. Neither this product nor materials prepared used by the T7 expression system are allowed to be distributed in the US and its territories without license of BSA. Information about license regarding the T7 expression system maybe obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 185, P.O. Box 500, Upton, New York 11973-5000, USA.