

## Product Information

### BL21(DE3)-T1<sup>R</sup> Competent Cells, Uni-Pack

Catalog Number **B2935**

Storage Temperature  $-70\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

BL21(DE3)-T1<sup>R</sup> competent *Escherichia coli* is an expression strain suitable for high level induction and expression of genes from any T7 promoter-based expression vector. BL21(DE3)-T1<sup>R</sup> is derived from a B/r strain bacterium<sup>1</sup> which naturally lacks the *lon* protease<sup>2</sup> and is also deficient in the outer membrane protease *ompT*. The absence of proteolytic activity from these two proteases may reduce degradation of some heterologous proteins expressed in the strain. The DE3 designation<sup>3-5</sup> indicates the strain is lysogenic for a lambda prophage containing an inducible T7 RNA polymerase, which is under the control of the *lacUV5* promoter. T7 RNA polymerase expression is induced by addition of 1 mM IPTG to the culture. In addition, the *tonA* genotype confers resistance to the lytic bacteriophages T1 and T5 for protection of clonal stocks.<sup>6</sup>

Sigma's BL21(DE3)-T1<sup>R</sup> competent *E. coli* cells are grown and made chemically competent using an optimized procedure specific to the strain, followed by strain verification and efficiency testing. The cells are provided in frozen 50  $\mu\text{L}$  aliquots for convenience. Each aliquot can be used for a single transformation. The cells have a transformation efficiency of  $>1 \times 10^7$  cfu/ $\mu\text{g}$  when transformed with non-saturating amounts of pUC19 plasmid DNA.

### Genotype of BL21(DE3)-T1<sup>R</sup>:

F<sup>-</sup> *ompT hsdS<sub>B</sub>(r<sub>B</sub><sup>-</sup> m<sub>B</sub><sup>-</sup>) gal dcm  $\lambda$ (DE3) tonA*

### Components/Reagents

BL21(DE3)-T1<sup>R</sup> Competent Cells are packaged with sufficient reagents for 10 transformation reactions.

- BL21(DE3)-T1<sup>R</sup> Competent Cells, 10 x 50  $\mu\text{L}$ , Catalog No. B3060
- pUC19 Control DNA, 10 ng/ $\mu\text{L}$ , 10  $\mu\text{L}$ , Catalog No. D2567

### Reagents and Equipment Required but Not Provided

- Shaker incubator (37  $^{\circ}\text{C}$ )
- Cabinet incubator (37  $^{\circ}\text{C}$ )
- Heated water bath (37  $^{\circ}\text{C}$ )
- SOC Medium, Catalog No. S1797
- LB Agar EZMix™ Powder, Catalog L7533
- LB Broth EZMix™ Powder, Catalog No. L7658
- Appropriate selection antibiotic
- 15 ml polypropylene culture tubes (sterile)
- Culture dishes
- Sterile Spreaders, Catalog No. Z376779

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

All components are stable for at least six months after receipt when properly stored at  $-70\text{ }^{\circ}\text{C}$ .

## Procedure

### Handling Tips:

1. Verify that the cells are still frozen and dry ice is still present in the shipping container upon receipt.
2. Handle the tubes as little as possible to prevent accidental warming of the cells. Keep cells on ice at all times.
3. When mixing the cells, gently swirl or tap the reaction tube. Do not mix by pipetting or vortexing.
4. Cells can be refrozen on dry ice and returned to  $-70\text{ }^{\circ}\text{C}$ , however transformation efficiency will decrease significantly with each freeze-thaw cycle.

### Before Starting:

1. Prepare LB agar plates with the appropriate antibiotic for selection of colonies that contain plasmid DNA, and warm to  $37\text{ }^{\circ}\text{C}$ .
2. Heat a water bath to  $37\text{ }^{\circ}\text{C}$ .

**Note:** Alternatively, the water bath may be set to  $42\text{ }^{\circ}\text{C}$ , but this variation on the procedure has been shown to be slightly less robust than  $37\text{ }^{\circ}\text{C}$ .

3. Warm SOC medium to room temperature ( $20\text{--}25\text{ }^{\circ}\text{C}$ ).

### I. Standard Transformation Protocol In Brief

**Note:** See Section II for detailed protocol

1. Thaw the required number of tubes containing cells on wet ice.
2. Add 1–50 ng of DNA (or 1  $\mu\text{L}$  of control DNA) to cells and gently tap the tube to mix.
3. Place cells on wet ice for 30 minutes.
4. Heat shock cells by incubating tubes in a  $37\text{ }^{\circ}\text{C}$  water bath for **exactly** 45 seconds.
5. Return the cells to ice for 2 minutes.
6. Add 450  $\mu\text{L}$  of SOC, at room temperature, to each transformation reaction.

7. Shake at 225 rpm at  $37\text{ }^{\circ}\text{C}$  for 1 hour.
8. Use a sterile spreader to evenly distribute transformation reaction over LB agar plates containing the appropriate selection antibiotic.
9. Incubate plates overnight at  $37\text{ }^{\circ}\text{C}$ .
10. If transformation was performed using purified plasmid DNA, select a colony and culture as detailed in the Expression Protocol (Section III).

### II. Detailed Standard Transformation Protocol

**Note:** DNA in ligation reactions can be added directly to Sigma's competent cells. Inactivation of the ligase is not required prior to transformation. No more than 50 ng of ligation reaction should be used in a 50  $\mu\text{L}$  transformation reaction.

Plasmid DNA isolated using miniprep procedures is typically satisfactory. To achieve maximum efficiency, the sample DNA should be free of phenol, ethanol, salts, protein, and detergents, and be dissolved in TE buffer or water.

1. Remove the required tubes of cells from the  $-70\text{ }^{\circ}\text{C}$  freezer, including one extra for the control DNA if desired. Place tubes immediately on wet ice so that only the cap is visible above the ice. Allow the cells to thaw on ice for approximately 5 minutes.
2. Visually examine cells to ensure they are thawed, and gently tap the vial several times to resuspend cells.
3. (Optional) Add 1  $\mu\text{L}$  pUC19 Control DNA (10 ng) to one tube of cells. Mix gently by tapping the tube. Return the cells to the ice.
4. Add 1–50 ng ligation reaction or purified plasmid DNA directly to cells. Mix as in Step 3.
5. Incubate the cells on wet ice for 30 minutes.
6. Heat shock the cells by incubating tubes in a  $37\text{ }^{\circ}\text{C}$  water bath for **exactly** 45 seconds.
7. Return the cells to ice immediately for 2 minutes.

8. Add 450  $\mu\text{L}$  of SOC medium, at room temperature, to each tube containing the cell/DNA mixture. Optimal recovery may be achieved by transferring the cells to a sterile 15 ml polypropylene culture tube. The cap should be loose to ensure sufficient air exchange and aeration of the culture.
9. Incubate cells at 37 °C with shaking (225-250 rpm) for 1 hour.
10. Transfer 10-100  $\mu\text{L}$  of each transformed cell suspension onto LB agar plates containing selection antibiotic and evenly distribute using a sterile spreader. Plates should be pre-warmed to 37 °C for optimal transformation efficiency. When inoculating less than 25  $\mu\text{L}$  of cell suspension, first pipette a drop of SOC onto the plate and add cell suspension to the SOC.
3. Incubate cells at 37 °C with shaking (225-250 rpm) until  $\text{OD}_{600}$  is between 0.2 and 0.5 (log phase).
4. Induce the cells by adding 100  $\mu\text{L}$  of 0.5 M IPTG to each culture. (Final IPTG concentration of 1.0 mM).
5. Incubate for an additional 3 hours at 37 °C with shaking. Time points may be taken to analyze expression.
6. Analyze cells by SDS-PAGE, western blot or enzymatic assay to determine if the protein of interest is being expressed.

Note: The amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. When using the control DNA, add no more than 10  $\mu\text{L}$  into a drop of SOC on an LB agar plate containing 100  $\mu\text{g}/\text{ml}$  ampicillin or carbenicillin.

11. If transformation was performed using purified plasmid DNA, select a colony and culture as detailed in the Expression Protocol (Section III).

### III. Expression Protocol<sup>7</sup>

**Note:** If you have an expression protocol for the plasmid you are working with, it is recommended that you use your own protocol. This protocol is intended only to provide general guidelines for induction with IPTG.

1. Pick several well-isolated colonies from each transformation reaction, and culture in 3 ml LB Broth containing the appropriate antibiotic for your plasmid. Shake at 37 °C overnight.
2. The following day, inoculate 1:100 in 50 ml LB Broth containing the appropriate antibiotic. There should be two cultures for each colony tested (one for an un-induced control).

### References

1. Wood, W. B., Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. *J. Mol. Biol.*, **16**, 118-133 (1966).
2. Phillips, T. A., *et al.*, lon gene product of *Escherichia coli* is a heat-shock protein. *J. Bacteriol.*, **159**, 283-287 (1984).
3. Studier, F. W., and Moffatt, B. A., Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.*, **189**, 113 (1986).
4. Rosenberg, A. H., *et al.*, Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene*, **56**, 125 (1987).
5. Studier, F. W., *et al.*, Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**, 60-89 (1990).
6. Braun, V., and Hantke, K., Bacterial receptors for phages and colicins as constituents of specific transport system, in *Receptors and Recognition*, Series B, Vol. 3, J. L. Reissig (Ed.), pp. 101-130. (Chapman & Hall, Ltd., London, 1977).
7. Sambrook, J. F., and Russell, D. W., *Molecular Cloning: A Laboratory Manual*, Third Ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

### Troubleshooting Guide

Problem	Cause	Solution	
No Colonies on plate	Cells thawed and re-frozen due to improper handling	Obtain new product, Temperature of ultracold freezer should be $-70^{\circ}\text{C}$ or colder	
	Old or expired cells	Obtain new product	
	No plasmid DNA added	Ensure plasmid DNA was added to cells	
	Input plasmid DNA missing antibiotic resistance ORF and/or replication of origin	Review cloning strategy	
	Low ligation efficiency	Plate increased volume of transformation reaction	
	Too much, or wrong antibiotic used	Adjust antibiotic levels in plates. Test a plate by streaking with an antibiotic resistant culture	
	Low efficiency transformation		Check to make sure transformation protocol was followed exactly
			Plate increased volume of transformation reaction
Check efficiency using included pUC19 control			
Too Many Colonies on Plate or a Lawn of Growth is Observed.	High efficiency transformation or high amounts of input DNA	Plate decreased volume of transformation reaction	
	Too little antibiotic	Adjust antibiotic levels in plates	
	Expired antibiotic	Use fresh antibiotic in plates	
	Contaminated SOC media	Obtain fresh medium and maintain sterile technique	
Unexpected Growth on Plate (i.e. fungal or mold)	<i>Contaminated plates</i>	Prepare and spread plates in laminar flow hood to reduce contamination	
	Contaminated SOC media	Obtain fresh medium and maintain sterile technique	

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#### Academic and Non-Profit Laboratory Assurance Letter

The T7 system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of U.S. Patent No. 5,693,489 (expiration date, December 2, 2014) assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a nonexclusive license for the use of this technology, including the enclosed material, based upon the following assurances:

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2. No materials that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase, may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this license and agrees to be bound by its terms. This limitation applies to strains of BL21(DE3), BL21(DE3)pLysS, and BL21(DE3)pLysE, and any derivatives.

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