BL21(DE3) Competent Cells, BL21(DE3)pLysS Competent Cells, and BL21 Competent Cells

Instruction Manual

Catalog #200131 (BL21(DE3) Competent Cells),
#200132 (BL21(DE3)pLysS Competent Cells), and
#200133 (BL21 Competent Cells)

Revision B

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MATERIALS PROVIDED

Material provided	Tube color[a]	Catalog number			Efficiency (cfu/μg of pUC18 DNA)[b]
		#200131	#200132	#200133	
BL21(DE3) competent cells	Green	5 × 0.2 ml	—	—	≥1 × 10^6
BL21(DE3)pLysS competent cells	Yellow	—	5 × 0.2 ml	—	≥1 × 10^6
BL21 competent cells	Blue	—	—	5 × 0.2 ml	≥1 × 10^6
pUC18 control plasmid[c]	—	10 μl	10 μl	10 μl	—
β-Mercaptoethanol (1.42 M)	—	25 μl	25 μl	25 μl	—

[a] The competent cells are packaged in color-coded tubes for convenience.

[b] These competent cell efficiencies are guaranteed when cells are used according to the specifications outlined in this instruction manual.

[c] The pUC18 control plasmid is shipped at a concentration of 0.1 ng/μl in TE buffer (see *Preparation of Media and Reagents*).

STORAGE CONDITIONS

Competent Cells: –80°C. Place the competent cells at –80°C immediately upon arrival. **Do not store the competent cells in liquid nitrogen.**

Revision B

ACADEMIC AND NONPROFIT LABORATORY ASSURANCE LETTER

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INTRODUCTION

Host Strains and Genotypes

For the *Escherichia coli* strains, the genes listed in the table below signify that the bacterium carries a mutant allele.

Host strain	Reference	Genotype
BL21 strain[a]	1	*E. coli* B F^- *dcm ompT hsdS*(r_B^- m_B^-) *gal*
BL21(DE3) strain[a]	1	*E. coli* B F^- *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)
BL21(DE3)pLysS strain[a]	1	*E. coli* B F^- *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3) [pLysS Cam^r]

[a] As *E. coli* B strains, these strains are general protein expression strains that lack both the *lon* protease and the *ompT* outer membrane protease, which can degrade proteins during purification.[2]

Features

We offer three different strains that provide varying levels of expression control with T7 promoter-driven vectors, such as the pCAL vectors‡ and the pET vectors‡. The BL21(DE3) competent cells are an all-purpose strain for high-level protein expression and easy induction. The BL21(DE3)pLysS competent cells provide tighter control of protein expression for expression of toxic proteins and are resistant to chloramphenicol. When used with the CE6 bacteriophage, the BL21 cells provide the tightest control of protein expression (see *BL21(DE3) Strains and Protein Toxicity*). The following table illustrates features of the BL21-derived expression strains for protein expression.

‡ Available separately from Agilent. Visit http://www.genomics.agilent.com for details.

Expression strain	Induction Method	Advantages	Disadvantages
BL21(DE3) competent cells	Isopropyl-1-thio-β-D-galactopyranoside (IPTG) induction of T7 polymerase from *lacUV5* promoter	High-level expression	Leaky expression of T7 polymerase can lead to uninduced expression of potentially toxic proteins
BL21(DE3)pLysS competent cells	Isopropyl-1-thio-β-D-galactopyranoside (IPTG) induction of T7 polymerase	Ease of induction	Slight inhibition of induced expression when compared with BL21(DE3)
BL21 competent cells	Infection with lambda bacteriophage CE6	Tightest control of uninduced expression	Induction not as efficient as DE3 derivatives
			Induction (infection) process more cumbersome

TRANSFORMATION GUIDELINES

Important *For optimal transformation efficiency, please read the guidelines outlined in the following sections before proceeding with the* Transformation Protocol.

Storage Conditions

The competent cells are very sensitive to slight variations in temperature. Storing the competent cells at the bottom of a –80°C freezer directly from the dry ice shipping container is required in order to prevent a loss of transformation efficiency. Transferring tubes from one freezer to another may result in a loss of efficiency. The transformation efficiency of the competent cells is guaranteed when the competent cells are used according to the specifications outlines in this instruction manual.

Use of 14-ml BD Falcon Polypropylene Round-Bottom Tubes

It is important to use 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) for the *Transformation Protocol* because the critical incubation period during the heat pulse is optimized specifically for the thickness and shape of these tubes.

Aliquoting Cells

Store the competent cells on ice at all times while aliquoting. It is essential to place the 14-ml polypropylene tubes on ice before the competent cells are thawed and to aliquot the competent cells directly into the prechilled 14-ml polypropylene tubes. It is also important to use 100 µl of competent cells/transformation. Using an inadequate volume of competent cells results in lower transformation efficiencies.

Use of β-Mercaptoethanol

β-Mercaptoethanol increases transformation efficiency two- to threefold. Use 1.7 µl of β-mercaptoethanol provided with this kit or a fresh 1:10 dilution of a 14.2 M stock solution per 100 µl of cells.

Quantity of DNA Added

Greatest efficiencies (i.e., transformants per microgram of DNA) are observed when adding 1 µl of ligated DNA at a concentration of 0.1 ng/µl per 100 µl of competent cells. Although the overall transformation efficiency may be lower, a greater number of colonies will be obtained when transforming up to 50 ng.

Length of the Heat Pulse

Optimal transformation efficiencies are observed when transformation reactions are heat-pulsed for 45–50 seconds. Transformation efficiencies decrease sharply when heat-pulsed for <45 seconds or for >60 seconds.

TRANSFORMATION PROTOCOL

1. Thaw the competent cells on ice.

   Note *Store the competent cells **on ice at all times** while aliquoting. It is essential that the 14-ml BD Falcon polypropylene round-bottom tubes are placed on ice before the competent cells are thawed and that 100 µl of competent cells are aliquoted directly into each **prechilled** polypropylene tube. Pipet the remaining competent cells into 100-µl aliquots and freeze the aliquots at –80°C. Do not pass the frozen competent cells through more than one freeze–thaw cycle.*

2. Gently mix the competent cells. Aliquot 100 µl of the competent cells into the appropriate number of 14-ml BD Falcon polypropylene round-bottom tubes. Prepare an additional 100-µl aliquot of cells for use as a transformation control.

3. Add 1.7 µl of the β-mercaptoethanol (β-ME) provided with this kit or a fresh 1:10 dilution of a 14.2 M stock solution of β-ME (diluted in distilled water), to each polypropylene tube containing the competent cells, for a final concentration of 25 mM β-ME. Swirl the tubes gently.

4. Incubate the reactions on ice for 10 minutes, swirling gently every 2 minutes.

5. Add 1–50 ng of ligated DNA to each transformation reaction and swirl gently. For the control transformation reaction, add 1 µl of the pUC18 control plasmid to a separate 100-µl aliquot of the competent cells and swirl gently.

6. Incubate the reactions on ice for 30 minutes.

7. Preheat SOC medium (see *Preparation of Media and Reagents*) in a 42°C water bath for use in step 10.

8. Heat-pulse each transformation reaction in a 42°C water bath for 45 seconds. **The duration of the heat pulse is critical for optimal transformation efficiencies.**

9. Incubate the reactions on ice for 2 minutes.

10. Add 0.9 ml of preheated (42°C) SOC medium to each transformation reaction and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.

11. Using a sterile spreader, spread ≤ 200 µl of the cells transformed with the experimental DNA onto LB agar[§] plates that contain the appropriate antibiotic.[‖]

    For the pUC18 control transformation, use a sterile spreader to plate 200 µl of the reaction onto an LB–ampicillin agar plate.[§]

12. Incubate the plates overnight at 37°C. The expected colony numbers for the control transformation reactions are as follows:

Host strain	Amount of transformation plated	Expected colony number	Efficiency (cfu/µg of pUC18 DNA)
BL21(DE3) strain	200 µl	>20	$\geq 1 \times 10^6$
BL21(DE3)pLysS strain	200 µl	>20	$\geq 1 \times 10^6$
BL21 strain	200 µl	>20	$\geq 1 \times 10^6$

INDUCTION OF TARGET PROTEIN USING IPTG

The following induction protocol is a general guide for expression of genes under the control of IPTG-inducible promoters on an analytical scale (1 ml of induced culture). Most commonly, this protocol is used to analyze protein expression of individual transformants when using BL21(DE3) host strains in combination with plasmids containing T7 promoter constructs (e.g. pET vectors). Expression cassettes under the control of the *trp/lac* hybrid promoter, *tac*, can be also induced using this protocol. In the case of *tac* promoter constructs, non-DE3 lysogen strains can be employed as hosts.

Note *The transformation procedure described above will produce varying numbers of colonies depending on the transformation efficiency obtained for the expression plasmid. It is prudent to test more than one colony as colony-to-colony variations in protein expression are possible.*

[§] See *Preparation of Media and Reagents*.

[‖] When spreading bacteria onto the plate, tilt and tap the spreader to remove the last drop of cells. If plating <100 µl of the transformation reaction, first place a 200-µl pool of SOC medium on the plate, pipet the cells into the pool of SOC, and then spread the mixture. If plating ≥100 µl, the cells can be spread directly onto the plates.

1. Inoculate 1 ml aliquots of LB broth containing the antibiotic required to maintain the expression plasmid with single colonies from the transformation. Shake at 220–250 rpm at 37°C overnight.

   Note *For the BL21(DE3)pLysS host strain, the overnight culture must contain chloramphenicol at a final concentration of 50 µg/ml in addition to the antibiotic required to maintain the expression plasmid. Chlorampenicol serves to maintain the pACYC-based plasmid carrying a T7 lysozyme gene derivative.*

2. The next morning, pipet 50 µl of each culture into fresh 1-ml aliquots of LB broth containing no selection antibiotics. Incubate these cultures with shaking at 220–250 rpm at 37°C for 2 hours.

3. Pipet 100 µl of each of the cultures into clean microcentrifuge tubes and place the tubes on ice until needed for gel analysis. These will serve as the non-induced control samples.

4. To the rest of the culture in each tube add IPTG to a final concentration of 1 mM. Incubate with shaking at 220–250 rpm at 37°C for 2 hours.

   Note *These values for IPTG concentration and induction time are starting values only and may require optimization depending on the gene expressed.*

5. After the induction period, place the cultures on ice.

6. Pipet 20 µl of each of the induced cultures into clean microcentrifuge tubes. Add 20 µl of 2× SDS gel sample buffer (see *Preparation of Media and Reagents*) to each microcentrifuge tube.

7. Mix the non-induced samples held on ice to resuspend the cells. Pipet 20 µl from each tube into a clean microcentrifuge tube. Add 20 µl of 2× SDS gel sample buffer to each of the 20-µl aliquots of cells.

8. Heat all tubes to 95°C for 5 minutes. Load the associated non-induced and induced samples in adjacent lanes for analysis by SDS-PAGE. Stain the protein gel with Coomassie® Brilliant Blue stain.

INDUCTION OF TARGET PROTEIN BY INFECTION WITH LAMBDA CE6

Expression of genes under the control of the T7 promoter (e.g. genes in pET vectors) can be achieved in non-DE3 lysogen host strains (e.g. BL21) if the strain harboring the expression plasmid is subsequently infected with lambda CE6. Lambda CE6 expresses T7 polymerase, which in turn drives the transcription of the gene downstream of the T7 promoter. The following protocols describe the growth and maintenance of lambda CE6 and the use of lambda CE6 for infecting host strains. We offer the Lambda CE6 Induction Kit (Catalog #235200) for use in protein expression protocols that incorporate CE6 infection.

Growth and Maintenance of High-Titer Bacteriophage Lambda CE6 Stocks

1. Inoculate 5 ml of modified* NZY broth§ with a single colony of LE392 host cells. Shake overnight at 37°C at 220–250 rpm.

2. Centrifuge the overnight culture for 15 minutes at 1700–2000 × *g* at 4°C. Resuspend the cells in 10 mM $MgSO_4$ to a final OD_{600} of 0.5.

3. Combine 250 µl of cells (at OD_{600} = 0.5) with 1 × 10^6 pfu of lambda CE6 in 14-ml BD Falcon polypropylene round-bottom tubes in triplicate. Incubate at 37°C for 15 minutes.

4. Add 3 ml of melted NZY top agar§ to each cell suspension and plate on warm agarose plates.§ Incubate the plates overnight at 37°C.

5. Flood each plate with 5 ml of SM solution§ and rock the plates for 2 hours at room temperature.

6. Remove the SM solution (which contains the lambda CE6) from each plate and pool the volumes in a 50-ml conical tube.

7. Centrifuge the SM solution at 1700–2000 × *g* for 15 minutes at 4°C.

8. Remove the supernatant and determine the titer of the solution.

9. Store the lambda CE6 stock at 4°C.

Phage Amplification

If the titer drops over time, or if more phage are needed, grow up LE392 cells in 10 ml of medium and add bacteriophage lambda CE6 at a multiplicity of infection of 1:1000 (lambda CE6-to-cell ratio). Continue growing the culture at 37°C for 5–6 hours and spin down the cellular debris. Titer of the supernatant should be ≥5.0 × 10^9 pfu/ml. For general information regarding phage amplification, see reference 3.

Induction of Target Protein by Infection with Lambda CE6

Note *This protocol is designed for induction in 50-ml culture volumes. If induction of a larger volume of culture is desired, it will be necessary to increase the volume of the overnight culture in step 1. The increased volume of overnight culture is necessary to achieve the required cell density ($A_{600} \leq 1$) in the larger volume of broth the following day.*

* NZY broth to be used for lambda infection protocols should be supplemented with maltose at a final concentration of 0.2%. Add 1 ml of 20% maltose solution (filter-sterilized) per 100 ml of NZY broth to achieve the correct final concentration of maltose.

§ See *Preparation of Media and Reagents*.

1. Inoculate 5 ml of modified* NZY broth containing the antibiotic required to maintain the expression plasmid with a single colony of BL21 cells (not the DE3 lysogen) harboring the expression plasmid. Shake overnight at 37°C at 200–250 rpm.

2. In the morning, centrifuge 1.0 ml of the overnight culture, resuspend the cells in 1.0 ml of fresh modified* NZY broth, and pipet the resuspended cells into a flask containing 50 ml of fresh modified* NZY broth (no selection antibiotics).

3. Record the A_{600} of the diluted culture. It should be ≤ 0.1. If the A_{600} is > 0.1, use more fresh modified NZY broth to dilute the culture to $A_{600} \leq 0.1$. If the A_{600} is < 0.1, the time required to reach an A_{600} of 0.3 (in step 4) will be extended.

4. Grow the culture to an A_{600} of 0.3 and then add glucose to a final concentration of 4 mg/ml (e.g. 1.0 ml of a 20% glucose solution to the 50-ml culture).

5. Grow the culture to an A_{600} of 0.6–1.0 and then add $MgSO_4$ to a final concentration of 10 mM (e.g. 500 µl of a 1.0 M solution of $MgSO_4$ to the 50-ml culture).

6. Remove a portion of the culture to serve as the uninduced control and infect the rest with bacteriophage lambda CE6 at a multiplicity of infection (MOI) of 5–10 particles per cell. (To optimize induction, cultures may be split into 3 or 4 aliquots and infected with varying dilutions of bacteriophage lambda CE6. The subsequent induction can be monitored by SDS-PAGE or by a functional assay, if available.)

7. Grow the culture for 2–3 hours after infection with lambda CE6.

8. Remove 5–20 µl of the culture for determination by SDS-PAGE, and harvest the remaining culture by centrifugation. Store the pellets at –70°C.

Note *If induction will be monitored using Coomassie stain, silver stain, or another nonspecific protein stain, we recommend running a control of CE6-infected BL21 cells harboring the plasmid without a cloned insert.*

* NZY broth to be used for lambda infection protocols should be supplemented with maltose at a final concentration of 0.2%. Add 1 ml of 20% maltose solution (filter-sterilized) per 100 ml of NZY broth to achieve the correct final concentration of maltose.

PREPARATION OF MEDIA AND REAGENTS

LB Broth (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
Add dH_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Cool to 55°C, and then add antibiotic, if required
Pour into petri dishes (~25 ml/100-mm plate)

SOB Medium (per Liter)

20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Autoclave
Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use

SOC Medium (per 100 ml)

Note *This medium should be prepared immediately before use*

1 ml of a 2 M filter-sterilized glucose solution **or** 2 ml of 20% (w/v) glucose
SOB medium to a final volume of 100 ml
Filter sterilize

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

2× SDS gel sample buffer

100 mM Tris-HCl (pH 6.5)
4% SDS (electrophoresis grade)
0.2% bromophenol blue
20% glycerol

Note *Add dithiothreitol to a final concentration in the 2× buffer of 200 mM prior to use.* ***This sample buffer is useful for denaturing, discontinuous acrylamide gel systems only.***

(table continues on following page)

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SM Solution 5 g of NaCl 2 g of $MgSO_4 \cdot 7H_2O$ 50 ml of 1 M Tris-HCl (pH 7.5) 5 ml 2% gelatin Add deionized H_2O to a final volume of 1 liter Adjust the pH to 7.5 Autoclave	**NZY Broth, (per Liter)** 5 g of NaCl 2 g of $MgSO_4 \cdot 7H_2O$ 5 g of yeast extract 10 g of NZ amine (casein hydrolysate) Add deionized H_2O to a final volume of 1 liter Adjust the pH to 7.5 with NaOH Autoclave
Agarose Plates (per Liter) Melt 20 g of agarose in 500 ml of deionized H_2O Add the following: 5 g of NaCl 5 g of yeast extract 10 g of tryptone Add deionized H_2O to a final volume of 1 liter Autoclave Pour into petri dishes (~25 ml/100-mm plate)	**NZY Top Agar (per Liter)** Prepare 1 liter of NZY broth Add 0.7% (w/v) agarose Autoclave Prior to use, melt agar in microwave, then hold at 48°C in water bath

REFERENCES

1. Weiner, M. P., Anderson, C., Jerpseth, B., Wells, S., Johnson-Browne, B. *et al.* (1994) *Strategies* 7(2):41–43.
2. Grodberg, J. and Dunn, J. J. (1988) *J Bacteriol* 170(3):1245-53.
3. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ENDNOTES

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MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.