Lambda CE6 Induction Kit

INSTRUCTION MANUAL

Catalog #235200
Revision A

For In Vitro Use Only
235200-12

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Lambda CE6 Induction Kit

MATERIALS PROVIDED

Materials provided	Quantity
Bacteriophage lambda CE6 (titer is ≥5.0 × 10^9 pfu/ml)	1.0 ml from high-titer lambda lysate stock
LE392 host cells (bacterial glycerol stock)	1.0 ml
BL21 competent cells	Five 0.2-ml tubes
pUC18 control plasmid[a]	10 µl
β-Mercaptoethanol (1.42 M)	25 µl

[a] 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

All Materials: –80°C

ADDITIONAL MATERIALS REQUIRED

14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059)

Revision A

ACADEMIC AND NONPROFIT LABORATORY ASSURANCE LETTER

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INTRODUCTION

The Lambda CE6 Induction Kit* is used to deliver T7 RNA polymerase into BL21 competent cells to induce expression of toxic proteins from T7 promoter–driven vectors, such as the pCAL and pET vectors. The Lambda CE6 Induction Kit allows tight control of protein expression, which is important when expressing toxic proteins since no T7 RNA polymerase is present in the cells until the desired time of induction. Bacteriophage lambda CE6 expresses T7 RNA polymerase from the lambda p_L and p_I promoters and carries the *Sam7* lysis mutations. T7 polymerase drives the transcription of the gene on the expression plasmid downstream of the T7 promoter. Bacteriophage lambda CE6 allows effective expression of target genes in BL21 cells and presumably other nonrestricting hosts which adsorb lambda. Lambda bacteriophage CE6 is propagated in the LE392 host strain,[1] which suppresses the *Sam7* mutation and consequently undergoes lysis.

HOST AND EXPRESSION STRAINS

LE392 Host Cells

e14$^-$*(mcrA) hsdR514 supE44 supF58 lacY1* or *Δ(laclZY)6 galK2 galT22 metB1 trpR55*

Streak bacterial glycerol stock on either LB or NZY agar plates (see *Preparation of Media and Reagents*).

BL21 Competent Cells

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

See reference 2.

* U.S. Patent No. 4,952,496.

AMPLIFYING THE LAMBDA CE6 BACTERIOPHAGE

It is important to titer the lambda CE6 bacteriophage prior to each use. Expect titers of approximately 5×10^9 pfu/ml. If the titer drops over time, prepare a fresh high-titer stock of the lambda bacteriophage as outlined in the amplification method below. For further details about lambda phage titering or amplification methods, see reference 3.

Plate Lysate Amplification Method

1. Transfer a single colony of LE392 cells into 5 ml of NZY broth with maltose.[§] Incubate with shaking at 37°C until growth reaches an OD_{600} of 1.0.

2. Centrifuge the overnight culture at 1700–2000 × *g* for 15 minutes at 4°C. Discard the supernatant. Resuspend the cell pellet 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 CE6 in 14-ml BD Falcon polypropylene round-bottom tubes in triplicate. Incubate the tubes at 37°C for 15 minutes without shaking to allow the phage to attach to the cells.

4. Add 3 ml of NZY top agar,[§] melted and cooled to ~48°C, to each cell suspension and plate on separate dry, prewarmed 100-mm agarose plates.[§] Allow the plates to set for 10 minutes. Invert the plates and incubate overnight at 37°C.

5. Overlay each plate with 5 ml of SM buffer.[§] Rock the plates for 4 hours at room temperature to allow the phage to diffuse into the SM buffer.

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

7. Add 500 µl of chloroform. Mix well and incubate for 15 minutes at room temperature.

8. Centrifuge the SM buffer at 1700–2000 × *g* for 15 minutes at 4°C to pellet the cell debris and chloroform.

9. Remove the phage-containing supernatant and determine the titer of the solution (see *Titering the Lambda CE6 Bacteriophage*). Store the lambda CE6 stock at 4°C.

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

TITERING THE LAMBDA CE6 BACTERIOPHAGE

1. Transfer a single colony of LE392 cells into 50 ml of NZY broth with maltose.

2. Incubate with shaking at 37°C for 4–6 hours (do not grow past an OD_{600} of 1.0). Alternatively, grow overnight at 30°C, shaking at 200 rpm.

   Note *The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.*

3. Pellet the bacteria at 1000 × *g* for 10 minutes.

4. Gently resuspend the cell pellet in sterile 10 mM $MgSO_4$ and dilute to an OD_{600} of 0.5.

   Note *The bacteria should be used immediately following dilution.*

5. Dilute aliquots of the lambda CE6 bacteriophage stock in SM buffer by the following amounts: 1:100, 1:1000, 1:10,000. Add 1 µl of each dilution to 200 µl of LE392 host cells at an OD_{600} of 0.5.

6. Incubate the phage and the bacteria at 37°C for 15 minutes to allow the phage to attach to the cells.

7. Add 3 ml of NZY top agar, melted and cooled to ~48°C, and plate immediately onto dry, prewarmed agarose plates. Allow the plates to set for 10 minutes. Invert the plates and incubate at 37°C.

8. Plaques should be visible after 6–8 hours. Count the plaques and determine the titer in plaque-forming units per milliliter (pfu/ml).

TRANSFORMATION GUIDELINES

Important *To achieve 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.

Aliquoting Cells

Store the competent cells on ice at all times while aliquoting. It is essential to place the BD Falcon polypropylene tubes on ice before the competent cells are thawed and to aliquot the competent cells directly into the prechilled BD Falcon 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 14-ml BD Falcon Polypropylene Tubes

It is important to use 14-ml BD Falcon polypropylene round-bottom tubes for the *Transformation Protocol*, because other tubes may be degraded by β-mercaptoethanol. Additionally, the critical incubation period during heat-pulsing is calculated for the thickness and shape (i.e., round bottom) of the BD Falcon polypropylene tubes.

Use of β-Mercaptoethanol

β-Mercaptoethanol increases transformation efficiencies 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 BL21 competent cells on ice.

   Note *Store the competent cells **on ice at all times** while aliquoting. It is essential that the BD Falcon polypropylene 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 prechilled 14-ml BD Falcon polypropylene tubes.

3. Add 1.7 µl of β-mercaptoethanol provided with this kit or a fresh 1:10 dilution of a 14.2 M stock solution of β-mercaptoethanol, diluted in distilled water (dH_2O), to each polypropylene tube containing the competent cells to a final concentration of 25 mM and swirl 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. 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.**

8. Incubate the reactions on ice for 2 minutes.

9. Add 0.9 ml of SOC medium (see *Preparation of Media and Reagents*) to each transformation reaction and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.

10. Using a sterile spreader, plate ≤200 µl of each transformation reaction directly onto separate LB agar plates containing the appropriate antibiotic. If plating ≥100 µl, the cells can be spread directly onto the plates. If plating <100 µl of the transformation reaction, plate into a 200-µl pool of SOC medium. When spreading bacteria onto the plate, tilt and tap the spreader to remove the last drop of cells.

    Use a sterile spreader to plate 200 µl of the control transformation reaction containing the pUC18 plasmid directly onto an LB–ampicillin agar plate (see *Preparation of Media and Reagents*). The expected results for the control transformation reaction are as follows:

Host strain	Transformation plated	Expected colonies	Efficiency (cfu/µg of pUC18 DNA)
BL21 strain	200 µl	>20	$\geq 1 \times 10^6$

INDUCTION OF TARGET PROTEIN BY INFECTION WITH BACTERIOPHAGE LAMBDA CE6

Note *This protocol is designed for inductions in 50-ml culture volumes. If inductions of larger volumes of culture are 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.*

1. Inoculate 5 ml of NZY broth with maltose containing the antibiotic required to maintain the expression plasmid with a single colony of BL21 cells harboring the expression plasmid. Shake overnight at 37°C at 200–250 rpm.

   Note *If the competent cells contain a pACYC-based plasmid (e.g., any BL21-CodonPlus strain), the overnight culture must include chloramphenicol at a final concentration of 50µg/ml in addition to the antibiotic required to maintain the expression plasmid.*

2. In the morning, centrifuge 1.0 ml of the overnight culture, resuspend the cells in 1.0 ml of fresh NZY broth with maltose, and pipet the resuspended cells into a flask containing 50 ml of fresh NZY broth with maltose.

3. Record the A_{600} of the diluted culture. It should be ≤ 0.1. If the A_{600} is >0.1, dilute the culture with fresh NZY broth with maltose to an A_{600} of ≤ 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 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 add $MgSO_4$ to a final concentration of 10 mM (e.g., 500 µl of 1.0 M $MgSO_4$ 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.

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 staining, silver staining, or another nonspecific protein staining methods, run a control of CE6-infected BL21 cells harboring the plasmid without a cloned insert.*

TROUBLESHOOTING

Observation	Suggestion
Problems associated with induction time	In certain cases, accumulation of target protein may kill cells at saturation while allowing normal growth in logarithmically growing cultures; in other cases, target protein may continue to accumulate in cells well beyond the recommended 3-hour induction period. To determine the optimal induction period, a time course may be performed in which a small portion of the culture is analyzed by SDS-PAGE at various times following induction
Inclusion bodies	In some cases, protein may form insoluble inclusion bodies at 37°C. In many cases, this protein may be soluble and active if the induction is carried out at 30°C. Inclusion body formation may be used as a purification step by simply spinning out the insoluble material from crude lysates and redissolving the protein in urea or guanidinium-HCl

PREPARATION OF MEDIA AND REAGENTS

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)

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Adjust the pH to 7.0 with 5 N NaOH
Add deionized H_2O to a final volume of 1 liter
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)

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)
Adjust the pH to 7.5 with NaOH

NZY Agar (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)
15 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave
Pour into petri dishes (~80 ml/150-mm plate)

NZY Top Agar (per Liter)

Prepare 1 liter of NZY broth
Add 0.7% (w/v) agarose
Autoclave

NZY Broth with Maltose

Prepare 1 liter of NZY broth
Autoclave
Add 3 ml of a 2 M filter-sterilized maltose solution or 10 ml of 20% (w/v) filter-sterilized maltose prior to use

SOB Medium (per Liter)

20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Autoclave
Add 10 ml of 1 M $MgCl_2$ and 10 ml of 1 M $MgSO_4$ prior to use
Filter sterilize

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

SM Buffer (per Liter)

5.8 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add deionized H_2O to a final volume of 1 liter
Autoclave

TE Buffer

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

REFERENCES

1. Phillips, T. A., VanBogelen, R. A. and Neidhardt, F. C. (1984) *J Bacteriol* 159(1):283-7.
2. Weiner, M. P., Anderson, C., Jerpseth, B., Wells, S., Johnson-Browne, B. *et al.* (1994) *Strategies* 7(2):41–43.
3. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MSDS INFORMATION

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