Novagen®

Bacteriophage CE6

Table of Contents

About the Kits

Bacteriophage CE6 Cloning Kit	0.2 ml	69390-3
	10 ml	69390-4

Description

Bacteriophage CE6 is a recombinant lambda phage containing the T7 RNA polymerase gene. The T7 RNA polymerase gene is cloned such that it is transcribed from the lambda p_L and p_I promoters during infection. In susceptible *E. coli* cells, λCE6 infection can be used to provide a source of T7 RNA polymerase for expressing genes from the T7 promoter of recombinant pET plasmids. λCE6 infection is an alternative to the use of a λDE3 lysogenic *E. coli* as host for inducible protein expression of a gene cloned in a pET vector. In general, λCE6 infection is used for protein expression when the cloned gene is so toxic that the plasmid cannot be maintained in DE3 lysogenic hosts, or with alternative hosts that are not available as DE3 lysogens.

Hosts for CE6-induced protein expression from pET plasmids must be susceptible to bacteriophage lambda infection. Lambda resistant *E. coli* (eg. λR, bar λ, /λ, *mal*B or *lam*B) cannot be used. However, neither *sup*F nor different immunity are required for T7 RNA polymerase synthesis and expression of cloned genes from pET vectors. Suitable strains for expression include BL21, B834, HMS174 and NovaBlue. BL21 and B834 are deficient for *omp*T and *lon* proteases. HMS174 and NovaBlue are *rec*A$^-$.

λCE6 is *int*$^-$*cI*$_{857}$S$_{am7}$ (Studier and Moffatt 1986). Because of the amber mutation in the S gene, *sup*F hosts are required for preparation of λCE6 stocks by lytic growth. It also cannot be propagated in *E. coli* that are lambda resistant or are lambda lysogens (lambda immunity). Because it is int$^-$, it is defective in formation of stable lysogens by integration into the *E. coli* chromosome. Host strains LE392 (r_K^- m_K^+) and ED8739 (r_K^- m_K^+) are suitable for propagation of λCE6.

The kit provides λCE6 as a high titer lysate. The 10 ml size provides sufficient phage to infect up to 100 ml of culture for protein expression. Host strains LE392 (r_K^- m_K^+) and ED8739 (r_K^- m_K^+) are included as glycerol stocks.

Components

- 0.2 or 10 ml Bacteriophage CE6 Lysate
- 0.2 ml LE392 glycerol stock
- 0.2 ml ED8739 glycerol stock

Storage

Store at –70°C.

Protocol

Expression of Target Genes

1. Prepare medium supplemented with 0.2% maltose and appropriate antibiotic to select for the pET plasmid. Inoculate with *E. coli* carrying the recombinant pET plasmid. Incubate at 37°C with shaking.

   Note: The use of minimal medium permits labeling of target protein with ^{35}S methionine. If a rich medium is preferred, LB or ZY broth can be used. Recipes are provided on page 4.
2. If using minimal medium, when OD_{600} of the culture reaches 0.3, add glucose to a final concentration of 4 mg/ml. For example, for the minimal medium described on page 4 (2 mg/ml glucose), add 1 ml 20% glucose to 100 ml culture to bring glucose up to 4 mg/ml.
3. Continue growing the cells for 1 to 2 h, or until the OD_{600} is between 0.6 and 1.0. Add $MgSO_4$ to a final concentration of 10 mM (eg. add 1 ml 1 M $MgSO_4$ to 100 ml culture).
   Note: The concentration of cells should be about 5 x 10^8/ml.
4. Add the λCE6 stock to a final concentration of 2–4 x 10^9 pfu/ml. Incubate 5 min without shaking.
   Note: The multiplicity of infection (MOI) should be between 5 and 10 phage per cell to ensure that almost every cell is infected, but to prevent the inhibition of protein synthesis that occurs at higher MOI.
5. Resume shaking at 37°C and continue growing the infected cells for 3 hr.
6. Harvest cells by centrifugation and purify target protein by desired method.

Preparation of λCE6 Stocks

The general procedures for the growth and storage of λCE6 are the same as those described for other bacteriophage lambda strains (Sambrook et al. 1989). The host strain for propagation of CE6 should be *supF* to supress the *Sam7* lysis mutation in the phage. The phage stock provided has been propagated on ED8739 (F^- *hsdS* (r_K^- m_K^-) *metB supE supF*), which lacks the *EcoK* restriction and modification systems. If the host for expression has an active *EcoK* restriction system, the phage should be propagated on a host which will provide the *EcoK* modification, such as LE392 (F^- *hsdR514* (r_K^- m_K^+) *mcrA supE44 supF58 lacY1* or Δ(*lacIZY*) *galK2 galT22 metB1 trpR55*).

The following protocol will generate 500 ml of a phage lysate with a titer of 10^9–10^{10} pfu/ml. The growth can be scaled up or down as needed.

1. Grow host cells (ED8739 or LE392) in LB supplemented with 0.2% maltose and 10 mM $MgSO_4$to an OD_{600} of about 1.0. Cells can be kept at 4°C until needed, but should be used within 2 days.
2. Mix 5 ml host cells with 2 x 10^8 phage particles. Incubate at 37°C for 15 minutes without shaking.
3. Add the host/phage mixture to 500 ml LB supplemented with 0.2% maltose and 10 mM $MgSO_4$. Use a 2.8 liter Fernbach flask (or Erlenmeyer flask) and shake at 250 rpm at 37°C until lysis is observed (usually within 5 h).
4. Add 5 ml chloroform to the flask and shake for 10 minutes.
5. Transfer to centrifuge bottle (leaving chloroform layer behind) and spin out the cell debris at 10,000 × *g* for 10 min at 4°C.
6. Decant the supernatant into a fresh bottle, taking precautions to avoid the cell pellet and any white chloroform layer.
7. Add dimethyl sulfoxide to final concentration of 7%.
8. Dispense the phage lysate into working aliquots (10–50 ml) and store at –70°C.
9. Determine the titer of the lysate by standard methods.

Recipes

Minimal Medium (per liter)	LB Broth (per liter)	ZY Broth (per liter)
6 g Na_2HPO_4 3 g KH_2PO_4 0.5 g NaCl 1 g NH_4Cl pH to 7.4 with 1 M NaOH autoclave, cool to < 60°C Add: 2 ml 1 M $MgSO_4$ 10 ml 20% glucose 0.1 ml 1 M $CaCl_2$	10 g Bacto-tryptone 5 g yeast extract 10 g NaCl pH to 7.5 with 1 M NaOH autoclave	10 g NZ amine A 5 g yeast extract 5 g NaCl autoclave
For plates, also add 15 g agar before autoclaving and 0.5 ml 1% thiamine after cooling.	For plates, also add 15 g agar before autoclaving,	For plates, also add 15 g agar before autoclaving.
Efficiency of lambda infection is increased if *E. coli* is grown in medium supplemented with 0.2% maltose. (Add 10 ml 20% maltose per L). Phage adsorption to *E. coli* requires Mg^{2+}. Add $MgSO_4$ to cultures to a final concentration of 10 mM before adding CE6.		

References

Hendrix, R.W., Roberts, J.W., Stahl, F.W. and Weisberg, R.A. (1983) *Lambda II* Cold Spring Harbor Laboratory.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory.

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