pEASY®-Blunt E2 Expression Kit

Please read the user manual carefully before use.

Cat. No. CE211

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Descriptions

pEASY®-Blunt E2 Expression Vector is constructed from pET vector. It utilizes a highly efficient, five-minute blunt cloning strategy to clone PCR product into high-efficient expression vector. The size of control insert is 750 bp, and expressed target protein is about 27 kDa.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- Ampicillin resistance.
- T7 promoter primer and T7 terminator primer for sequencing.
- Bacteriophage T7*lac* promoter for high level expression.
- C-terminal 6×His tag for easy purification.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.
- E2 Expression Plasmid included as negative control.

Kit Contents

Component	CE211-01 (10 rxns)
pEASY®-Blunt E2 Expression Vector (15 ng/μl)	10 μl
E2 Expression Plasmid (Negative Control) (15 ng/μl)	10 μl
EControl Template (5 ng/μl)	10 μl
EControl Forward Primer (10 μM)	10 μl
EControl Reverse Primer (10 μM)	10 μl
T7 Promoter Primer (10 μM)	50 μl
T7 Terminator Primer (10 μM)	50 μl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5×100 μl

Gene Cloning

Cloning reaction

(1) Primer requirement: primer cannot be phosphorylated

(2) PCR Enzyme: high fidelity *Pfu* DNA polymerase

(3) Reaction conditions: for higher cloning efficiency, we recommend 5-10 minutes post PCR 72°C extension. After PCR, use agarose gel electrophoresis to verify the quality and quantity of PCR product.

Setting Up the Cloning Reaction

Add following components into a microcentrifuge tube.

PCR products 0.5-4 μl (can be increased or reduced based on the amount of PCR product, but no more than 4 μl)

pEASY®-Blunt E2 Expression Vector 1 μl

Gently mix and incubate the mixture at room temperature (20°C-37°C) for 5 minutes. After reaction, place the tube on the ice.

1. Optimal amount of insert
   Molar ratio of vector to insert = 1:7 (1 kb, ~20 ng; 2 kb, ~40 ng)
2. Optimal volume of vector: 1 μl
3. Optimal reaction volume: 3~5 μl

4. Optimal incubation time
(1) 0.1~1 kb (including 1 kb): 5~10 minutes
(2) 1~2 kb (including 2 kb): 10~15 minutes
(3) 2~3 kb (including 3 kb): 15~20 minutes
(4) ≥3 kb: 20~30 minutes
Use the maximal incubation time if the insert is gel purified PCR product.
5. Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

1. Add the ligated products to 50 µl of *Trans*1-T1 phage resistant chemically competent cells and mix gently (do not mix by pipetting up and down).
2. Incubate on ice for 20-30 minutes.
3. Heat-shock the cells at 42°C for 30 seconds.
4. Immediately place the tube on ice for 2 minutes.
5. Add 250 µl of room temperature SOC or LB medium. Shake the tube at 37°C (200 rpm) for 1 hour.
6. Pre-warm a selective LB plate at 37°C for 30 minutes.
7. Spread 200 µl or all transformant on the pre-warmed plate. Incubate overnight at 37°C.

Analysis of positive clones

1. Transfer 5~10 colonies into 10 µl Nuclease-free Water.
2. Use 1 µl of the mixture as template for 25 µl PCR using T7 promoter primer and gene reverse primer, or gene forward primer and T7 terminator primer.
3. PCR

94°C	10 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	x min*	
72°C	5-10 min	

* (depends on the insert size and PCR enzymes)
4. Analyze plasmids by restriction enzyme disgestion or DNA sequencing.

Target gene expression

1. Competent cell
BL21(DE3) competent cell series are suitable for prokaryotic protein expression.
2. Protein expression
• Pick single colony and transfer into 5 ml of LB/Amp$^+$ medium and shake at 37°C (250 rpm) until OD_{600} close to 0.5.
• Add IPTG to a final concentration of 0.5-1 mM and shake at 37°C for 3-5 hours.
• For optimal result, try different IPTG concentration and inducing time.
3. Check expression
Aspirate the supernatant and use the pellets for SDS-PAGE.

Protein Purification

Refer to the section of *ProteinIso*® Ni-NTA Resin in user manual.

Notes

• The amount of PCR products used for cloning can be adjusted based on the yield and size of the product.
• Do not use more than 30 minutes for cloning reaction.

• Volume of the whole reaction system should not be more than 5 μl.
• Cloning efficiency declines with the increase in the size of cloned fragment (>3 kb).
• PCR purification kit is recommended for use if primer dimmers formed. Gel purification is recommended if multibands are present.

Troubleshooting

(1) Low cloning efficiency

Various factors can affect cloning efficiency, such as primers used in target gene amplification, ratio of insert fragment to vector, etc. If low cloning efficiency is observed, try to use following methods to increase cloning efficiency.

a. Purify the PCR product
b. Increase the volume of inserts for low concentration of inserts
c. Use fresh PCR products

(2) Unsuccessful identification of transformants by PCR

When PCR is used for identifying transformants, neither target amplification product nor self-ligation of vector is obtained, it indicates a failure in PCR reaction. Re-optimize PCR reaction condition or extract plasmids, then perform amplification with plasmid as template or identify transformants-containing clones by restriction enzyme digestion.

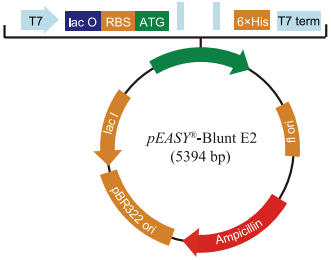
PCR for control insert (750 bp)

Component	Volume	Final Concentration
EControl Template (5 ng/μl)	1 μl	0.1 ng/μl
EControl Forward Primer (10 μM)	1 μl	0.2 μM
EControl Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransStart® FastPfu* PCR SuperMix	25 μl	1×
Nuclease-free Water	Variable	-
Total Volume	50 μl	-

PCR

94℃ 10 min
94℃ 30 sec
55℃ 30 sec
72℃ 1 min
30 cycles
72℃ 10 min

pEASY®- Blunt E2 Prokaryotic Expression Vector Map



T7 promoter: bases 5,117-5,133
T7 transcription start: bases 5,134
Lac operator(lacO): bases 5,136-5,160
RBS: bases 5,190-5,196
His·Tag coding sequence: bases 5,238-5,255
T7 terminator: bases 5,323-5,369
ROP ORF: bases 2,648-2,839
*Lac*I ORF: bases 3,651-4,739
pBR origin: bases 1,614-2,233
Ampicillin resistance ORF: bases 599-1,459
f1 origin: bases 13-450

T7 Promoter
T7 Transcription Start
lac operator
Xba I
TAA TAC GAC TCA CTA TAG GGG AAT TGT GAG CGG ATA ACA ATT CCC CTC TAG AAA TAA TTT
RBS
Nde I
Xho I
Polyhistidine region
TGTTTAACTTTAA GAAGGAGA TATA CAT ATG GAA TTG GCC CTT PCR Product AAG GGC CAA TTC CTC GAG CAC CAC CAC CAC CAC CAC TGAGATCCGGCTGCTAAC
GTA TAC CTT AAC CGG GAA TTC CCG Gln Phe Leu Glu His His His His His His ACTCTAGGCCGACGATTG
T7 Terminator Primer
T7 Terminator
AAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCAC CGCTGAGCAATAA CTAGCATAA CCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG CTGAAAGGAGGAACTATATCCGGAT

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