pEASY®-Blunt Zero Cloning Kit

Please read the user manual carefully before use.

Cat. No. CB501

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 Zero Cloning Vector contains a suicide gene. Ligation of PCR fragment disrupts the expression of the gene. Cells that contain non-recombinant vector are killed upon plating. Therefore, blue/white selection is not required.

- 5 minutes fast ligation of *Pfu*-amplified PCR products.
- High cloning efficiency. Positive clones up to 100%.
- No blue/white selection needed.
- Suitable for larger fragment cloning.
- Kanamycin and Ampicillin resistance genes for selection.
- M13 forward primer and M13 reverse primer for sequencing.
- T3 promoter and T7 promoter for *in vitro* transcription.
- *Trans*1-T1 Phage Resistant Chemically Competent Cell, high transformation efficiency (>10^9 cfu/μg pUC19 DNA) and fast growing.

Kit Contents

Component	CB501-01 (20 rxns)	CB501-02 (60 rxns)
pEASY ®-Blunt Zero Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
Trans 1-T1 Phage Resistant Chemically Competent Cells	10×100 μl	30×100 μl

Preparation of PCR Products

1. Primer requirement: primer cannot be phosphorylated
2. PCR Enzyme: *Pfu* DNA polymerases
3. Reaction conditions: in order to ensure the integrity of amplification products, 5-10 minutes of post-extension step is required. After amplification, use agarose gel electrophoresis to verify the quality and quantity of PCR product

Setting Up the Cloning Reaction System

Add following components into a microcentrifuge tube.

PCR products 0.5-4 μl (can be increased or reduced based on PCR product yield, not more than 4 μl)

pEASY ®- Blunt Zero CloningVector 1 μl

Gently mix well, incubate at room temperature (20°C-37°C) for 5 minutes, and then 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 (10 ng)
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 maximum incubation time if the insert is gel purified.

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 Cell 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. Spread 200 µl or all transformants on the pre-warmed plate. Incubate at 37°C overnight.

Identification of Positive Clones and Sequencing

Analysis of positive clones

1. Transfer 5~10 white or light blue colonies into 10 µl Nuclease-free Water and vortex.
2. Use 1 µl of the mixture as template for 25 µl PCR using M13 forward and M13 reverse primers.
3. PCR reaction conditions

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 positive clones by restriction enzyme digestion and DNA sequencing.
   Inoculate positive clones on LB/Amp$^+$ or LB/Kan$^+$ liquid medium, grow at 37°C for 6 hours at 200 rpm. Isolate plasmid DNA by plasmid MiniPrep Kit. Analyze plasmids by restriction enzyme digestion with proper restriction endonuclease.

Sequencing

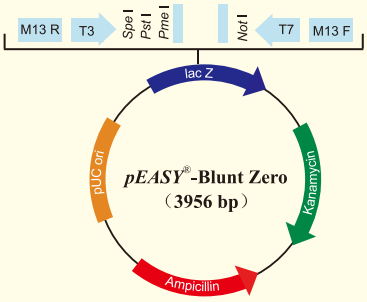
Analyze the sequence by sequencing with M13 F, M13 R and T7 promoter.

PCR for control insert (700 bp)

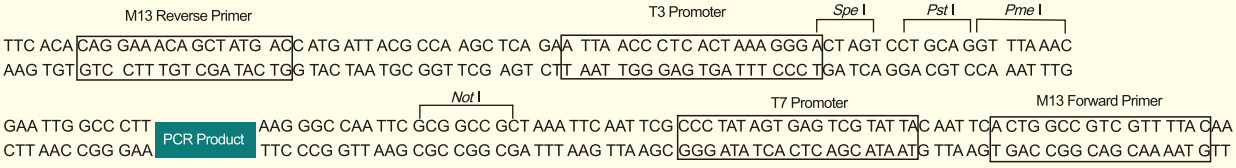
Component	Volume	Final Concentration
Control Template (5 ng/µl)	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2×*EasyPfu* PCR SuperMix	25 µl	1×
Nuclease-free Water	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

94℃	2~5 min	
94℃	30 sec	30 cycles
50~60℃	30 sec	
72℃	1 min	
72℃	10 min	



*LacZ*α fragment: bases 217-810
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 328-347
M13 Forward priming site: bases 354-370
Kanamycin resistance ORF: bases 1,159-1,953
Ampicillin resistance ORF (c): bases 2,203-3,063
pUC origin: bases 3,161-3,834
(c) = complementary strand



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