AllBio Blunt Zero Cloning Kit

Please read the user manual carefully before use.

Cat. No. ABTGMBZ501

Storage: AllBio T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Description

AllBio 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.
- Trans1-T1 phage resistant chemically competent cell, high transformation efficiency (>109 cfu/μg pUC19 DNA) and fast growing.

Kit Contents

Component	10 rxns
AllBio Blunt Zero Cloning Vector (10 ng/μl)	10 μl
Control Template (5 ng/μl)	2.5 μl
Control Primers (10 μM)	2.5 μl
M13 Forward Primer (10 μM)	25 μl
M13 Reverse Primer (10 μM)	25 μl
AllBio T1 Phage Resistant Chemically Competent Cell (Option) -70°C Cat. ABTGCD501-10	5x100 μl

Preparation of PCR Products

1. Primer requirement: primer cannot be phosphorylated
2. Enzyme choice: *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)

AllBio 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).

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Transformation

1. Add 5 µl of ligation products into 50 µl of AllBio T1 Chemically Competent Cell (ligation product should be added immediately after thawing the cells on ice) and mix by tapping gently. Incubate on ice for 20-30 minutes.
2. Heat shock at 42°C water bath for exactly 30 seconds, and place on ice for 2 minutes.
3. Add 250 µl of room temperature SOC or LB medium. Incubate at 37°C for 1 hour at 200 rpm.
4. Spread 200 µl transformants on the plate and incubate overnight (to obtain more colonies, centrifuge cells at 4000 rpm for 1 minute, discard a portion of supernatant and keep 100-150 µl of it. Gently tapping to suspend the cells, plate all the cells and incubate overnight).

Identification of Positive Clones and Sequencing

Identify Positive Clones by PCR

1. Pick several colonies and place them in 10 µl sterile water, mix by vortexing.
2. Add 1 µl mixture into 25 µl reaction volume. Identify positive clones with M13 forward primer and reverse primer.
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)

Analyzing Positive Clones by restriction enzyme digestion

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	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2×EasyPfu PCR SuperMix	25 µl	1X
ddH_2O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

94°C	2~5 min	
94°C	30 sec	30 cycles
50~60°C	30 sec	
72°C	1 kb/min	
72°C	10 min	

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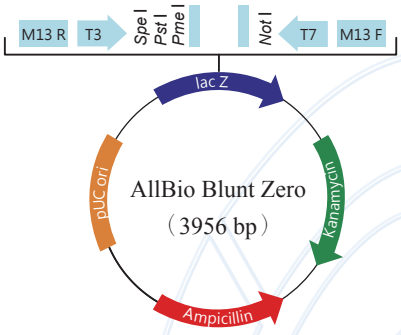

fragment: bases 217-810
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 328-347
M13 Forward priming site: bases 354-370
Kanamycin resistance ORF: bases 1159-1953
Ampicillin resistance ORF (c): bases 2203-3063
pUC origin: bases 3161-3834
(c) = complementary strand

M13 Reverse Primer
T3 Promoter
SpeI
Pst I
Pme I

TTC ACA CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TCA GAA TTA ACC CTC ACT AAA GGG ACT AGT CCT GCT GGT TTA AAC
AAG TGT GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AGT CTT AAT TGG GAG TGA TTT CCC TGA TCA GGA CGT CCA AAT TTG

Not I
T7 Promoter
M13 Forward Primer

GAA TTG GCC CTT PCR Product AAG GGC CAA TTC GCG GCC GCT AAA TTC AAT TCG CCC TAT AGT GAG TCG TAT TAC AAT TCA CTG GCC GTC GTT TTA CAA
CTT AAC CGG GAA TTC CCG GTT AAG CGC CGG CGA TTT AAG TTA AGC GGG ATA TCA CTC AGC ATA ATG TTA AGT GAC CGG CAG CAA AAT GTT