pEASY®-T1 Cloning Kit

Please read the user manual carefully before use.
Cat. No. CT101
Version No. Version 1.1
Storage: *Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C or below for one year; others at -18°C or below for one year.

Description

The *pEASY*®-T1 Cloning Vector contains the LacZ gene, enabling blue-white screening on plate media with IPTG and X-gal, making it suitable for TA cloning.

Features

Fast: Takes only 5 minutes.
Simple: Just add the fragment.
Efficient: High positive rate.
Provides two selection markers, ampicillin and kanamycin, allowing flexible selection based on experimental needs.
Multiple restriction enzyme sites for convenient subcloning.
Sequencing primers: M13 Forward Primer, M13 Reverse Primer, T7 Promoter Primer.
T7 Promoter for in vitro transcription.
Trans1-T1 competent cells offer high transformation efficiency and fast growth, ensuring high clone yield and reducing screening time.

Kit Contents

Component	CT101-01 (20 rxns)	CT101-02 (60 rxns)
pEASY ®-T1 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

1. Preparation of PCR Products

(1) Primer requirement: primer cannot be phosphorylated.
(2) PCR Enzyme: Taq 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 reaction, use agarose gel electrophoresis to verify the quality and quantity of PCR product. If the PCR product shows multiple bands, perform gel recovery to isolate the target fragment.

2.Setting Up the Cloning Reaction System

(1) Add following components into a microcentrifuge tube.

Component	Volume
PCR Product	0.5-4 μl
pEASY®-T1 Cloning Vector	1 μl

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

3. Recommended Cloning Reaction Conditions

• Optimal Insert DNA Amount
Vector to insert molar ratio = 1:7
A rough calculation can be made using the ratio "1 kb 20 ng" (e.g., add 20 ng for a 1 kb fragment, 30 ng for a 1.5 kb fragment, etc.).

• Optimal Vector Amount: 1 µl
• Optimal Reaction Volume: 3-5 µl; if the volume is insufficient, sterile water can be added.
• Optimal Reaction Time
(1) Insert length 0.1-1 kb (including 1 kb): 5-10 min*
(2) Insert length 1-2 kb (including 2 kb): 10-15 min*
(3) Insert length 2-3 kb (including 3 kb): 15-20 min*
(4) Insert length above 3 kb: 20-30 min*
*If the fragment is a gel recovery product, use the maximum reaction time.
• Optimal Reaction Temperature: 25°C; for high GC content fragments, the reaction can be performed at 37°C. (Temperature control using a PCR machine is recommended.)

4. Transformation

(1) Add the ligation product to 50 µl of Trans1-T1 competent cells (add the ligation product immediately after the cells have just thawed). Mix gently by flicking, and incubate on ice for 20–30 minutes.
(2) Heat shock at 42°C in a water bath for 30 seconds, then immediately place on ice for 2 minutes.
(3) Add 250 µl of room-temperature SOC or LB medium, and incubate at 37°C with shaking at 200 rpm for 1 hour.
(4) Mix 8 µl of 500 mM IPTG with 40 µl of 20 mg/ml X-gal, spread evenly onto prepared plates, and place in a 37°C incubator for 30 minutes.
(5) After the IPTG and X-gal have been absorbed, spread 200 µl of the bacterial culture evenly onto the plates, and incubate overnight at 37°C (to obtain more colonies, centrifuge at 1,500 × g for 1 minute, discard part of the supernatant, retain 100–150 µl, gently flick to resuspend the cells, and spread the entire suspension onto the plate).

Positive Clone Detection

1. Identification of Positive Clones by PCR
(1) Pick a single white colony and transfer it into 10 µl of sterile water. Mix by vortexing.
(2) Use 1 µl of this mixture in a 25 µl PCR reaction. Identify positive clones using M13 Forward Primer and M13 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	

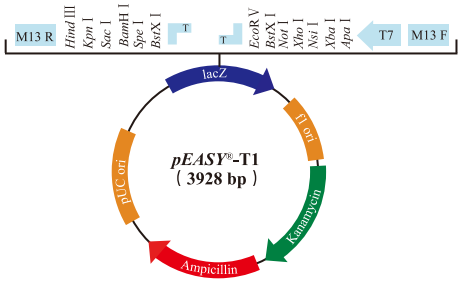
2. Identification of Positive Clones by Restriction Enzyme Analysis
Pick a single white colony and inoculate it into liquid LB medium containing Amp^+ or Kan^+. Incubate at 37°C with shaking at 200 rpm for approximately 6 hours. Use a kit to perform small-scale plasmid extraction, select appropriate restriction enzymes, and digest the plasmid to identify positive clones.
3. Identification of Positive Clones by Sequencing
Perform sequencing using M13 Forward Primer, M13 Reverse Primer, and T7 promoter primer for sequence analysis.

PCR for control insert (700 bp)

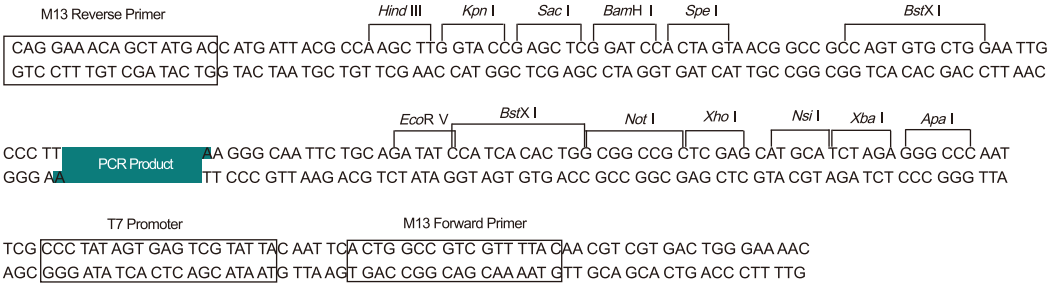
Component	Volume	Final Concentration
Control Template (5 ng/µl)	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2×*EasyTaq*® PCR SuperMix	25 µl	1×
Nuclease-free Water	Variable	-
Total volume	50 µl	-

Thermal cycling conditions for control insert

94°C	2-5 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	1 min	
72°C	10 min	



*LacZ*α fragment: bases 1-544
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-354
T7 promoter priming site: bases 361-380
M13 forward priming site: bases 387-403
f1 origin: bases 545-982
Kanamycin resistance ORF: bases 1,316-2,110
Ampicillin resistance ORF: bases 2,128-2,988
pUC origin: bases 3,133-3,806



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