pEASY®-T1 Simple Cloning Kit

Please read the user manual carefully before use.

Cat. No. CT111

Storage
Trans1-T1 Phage Resistant Chemically Competent Cell at -70°C for six months; others at -20°C for nine months

Descriptions
pEASY® - T1 Simple Cloning Vector eliminates the multi-cloning sites of pEASY®-T1 Cloning Vector. It is designed for cloning and sequencing Taq-amplified PCR products.
• 5 minutes fast ligation of Taq-amplified PCR products.
• Kanamycin and Ampicillin resistance genes for selection.
• Easy blue/white selection.
• SR primer and M13 forward primer for sequencing.
• T7 promoter for in vitro transcription.
• Trans1-T1 Phage Resistant Chemically Competent Cells, high transformation efficiency (>10⁹ cfu/µg pUC19 DNA) and fast growing.

Kit Contents

<table>
<thead>
<tr>
<th>Component</th>
<th>CT111-01 (20 rxns)</th>
<th>CT111-02 (60 rxns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pEASY®-T1 Simple Cloning Vector (10 ng/µl)</td>
<td>20 µl</td>
<td>3×20 µl</td>
</tr>
<tr>
<td>Control Template (5 ng/µl)</td>
<td>5 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>Control Primers (10 µM)</td>
<td>5 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>M13 Forward Primer (10 µM)</td>
<td>50 µl</td>
<td>150 µl</td>
</tr>
<tr>
<td>M13 Reverse Primer (10 µM)</td>
<td>50 µl</td>
<td>150 µl</td>
</tr>
<tr>
<td>SR Primer (10 µM)</td>
<td>50 µl</td>
<td>150 µl</td>
</tr>
<tr>
<td>Trans1-T1 Phage Resistant Chemically Competent Cell</td>
<td>10 × 100 µl</td>
<td>30 × 100 µl</td>
</tr>
</tbody>
</table>

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

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, no more than 4 µl)
pEASY®-T1 Simple Cloning Vector 1 µl
Gently mix well, incubate at room temperature (20°C-37°C) for 5 minutes. After reaction, place the tube on 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 Trans1-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. In the meantime, mix 8 μl of 500 mM IPTG with 40 μl of 20 mg/ml X-gal. Spread them evenly onto a selective LB plate. Place the plate at 37°C for 30 minutes.
7. 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 ddH₂O 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
   55°C 30 sec
   72°C x min*
   72°C 5–10 min
* (depends on the insert size and PCR enzymes) the PCR product size from vector self-ligation is 100 bp.
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 colonies 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)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Template (5 ng/μl)</td>
<td>1 μl</td>
<td>0.1 ng/μl</td>
</tr>
<tr>
<td>Control Primers (10 μM)</td>
<td>1 μl</td>
<td>0.2 μM</td>
</tr>
<tr>
<td>2×EasyTaq® PCR SuperMix</td>
<td>25 μl</td>
<td>1×</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Variable</td>
<td>-</td>
</tr>
<tr>
<td>Total volume</td>
<td>50 μl</td>
<td>-</td>
</tr>
</tbody>
</table>

Thermal cycling conditions for control insert
94°C 2.5 min
94°C 30 sec
55°C 30 sec 30 cycles
72°C 1 min
72°C 10 min

Ligate 1 μl of control PCR insert with 1 μl vector. Hundreds of colonies should be produced with cloning efficiency over 90%.
LacZa fragment: bases 1-445
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 262-281
M13 forward priming site: bases 288-304
fl origin: bases 446-883
Kanamycin resistance ORF: bases 1,217-2,011
Ampicillin resistance ORF: bases 2,029-2,889
pUC origin: bases 3,034-3,707