



# Asia Hepato Gene Co. Product Datasheet

華肝基因股份有限公司 website: <http://www.ah-gene.com.tw>  
806 高雄市前鎮區復興四路 12 號 8F-1 Tel : 07-9553811~62 Fax : 07-9558777

## pM-CT TA Cloning kit

### 10 Reactions

#### Application:

1. PCR product cloning
2. Protein expression in E. coli

#### Kit Components:

	Item	Concentration	Amount	Storage
1	pM-CT TA vector	<ul style="list-style-type: none"> <li>• Size: 2,700bp</li> <li>• 50ng/μl</li> <li>• IPTG induce Protein expression in E. coli</li> <li>• Ampicillin resistance</li> <li>• Contain ATG start codon</li> <li>• C-terminal Polyhistidine (6×His) tag for detection and purification</li> </ul>	25μl	-20°C
2	10x Ligation Buffer A	0.4M Tris-HCl, 0.1mM MgCl <sub>2</sub> , 0.1M DTT, 5mM ATP	10μl	-20°C
3	10x Ligation Buffer B	Enhance ligation efficiency	10μl	-20°C
4	Ligase	50mM KCl, 20mM Tris-HCl.(pH7.5).0.1mM EDTA, 1mM DTT, 50% glycerol	5μl	-20°C
5	SOC Medium	2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl <sub>2</sub> , 10 mM MgSO <sub>4</sub> , 20 mM glucose.	1.5ml	25°C
6	T7 Forward primer	10μM/μl in 1×TE buffer, pH8.0 ,Tm 50°C Sequence: 5'-TAATACGACTCACTATAGGG-3'	50μl	-20°C
7	T7 Term Reverse primer	10μM/μl in 1×TE buffer, pH8.0,Tm 50°C Sequence: 5'-ATCCGGATATAGTTCCTCCTTTC-3'	50μl	-20°C
8	DH5α competent cell	Transformation efficiency >3×10 <sup>8</sup> cfu/μg DNA	100μl×10	-80°C
9	pUC19 Control DNA	10 <sup>-4</sup> μg/μl	5μl	-20°C

#### PCR Primer Design:

Forward primer	place the gene of interest in frame, the first 3 nt with or without initiation ATG codon for protein expression.
Reverse primer	remove stop codon in the gene of interest and preserve the reading frame through the C-terminal 6×His tag.

*For research use only, not for diagnostic or therapeutic use.*

### High Yield PCR Product:

94°C → 94°C → X°C → 72°C → 72°C → 25°C  
5min 30sec 30sec 30sec 7min end  
30 cycles

Load 16µl PCR product to run 1-2% small wells agarose gel, target gene should be clearly visible on the gel

### Ligation:

1. Add as following and carefully flick the tube 5-6 times to mix. Do not vortex.  
\* PCR product contain Ampicillin resistance template must be purified by agarose gel to remove Ampicillin resistance template

pM-CT TA vector	2.5µl	2.5µl	2.5µl	2.5µl
PCR product <3kb	1µl	2µl	3µl	4µl
10x Ligation Buffer A	1µl	1µl	1µl	1µl
10x Ligation Buffer B	1µl	1µl	1µl	1µl
Ligase	0.5µl	0.5µl	0.5µl	0.5µl
dH2O	4µl	3µl	2µl	1µl
total	10µl	10µl	10µl	10µl

2. The optimal ligation efficiency molar ratio vector : insert = 1 : 3.  
Low yield and long PCR product need more.
3. Centrifuge briefly and incubate at room temperature (25°C) for 5-20 minutes will suffice for PCR product <3kb.
4. If PCR product >3kb, incubate at 4°C overnight

### Transformation:

1. Thaw DH5α competent cell at room temperature before use
2. Add 108µl competent cell into 10µl ligation mixture and mix gently.
3. On ice or 4°C for 10 minutes
4. 42°C 45 seconds
5. Add 80µl SOC Medium
6. Spread 168µl cells onto 20-50µg/ml Ampicillin plate. Excessive antibiotic significantly decreased the transformation efficiency
7. 37°C overnight

### Analyzing Positive Clones:

1. Pick 12 colonies or more and culture them overnight in LB containing 20-50µg/ml ampicillin.
2. Isolate plasmid DNA.
3. Analyze the plasmids by restriction analysis, sequencing,
4. PCR use **target gene forward primer** and **T7 Term Reverse primer** (add 0.5µl per 25µl PCR reaction) to confirm the presence and correct orientation of the insert.

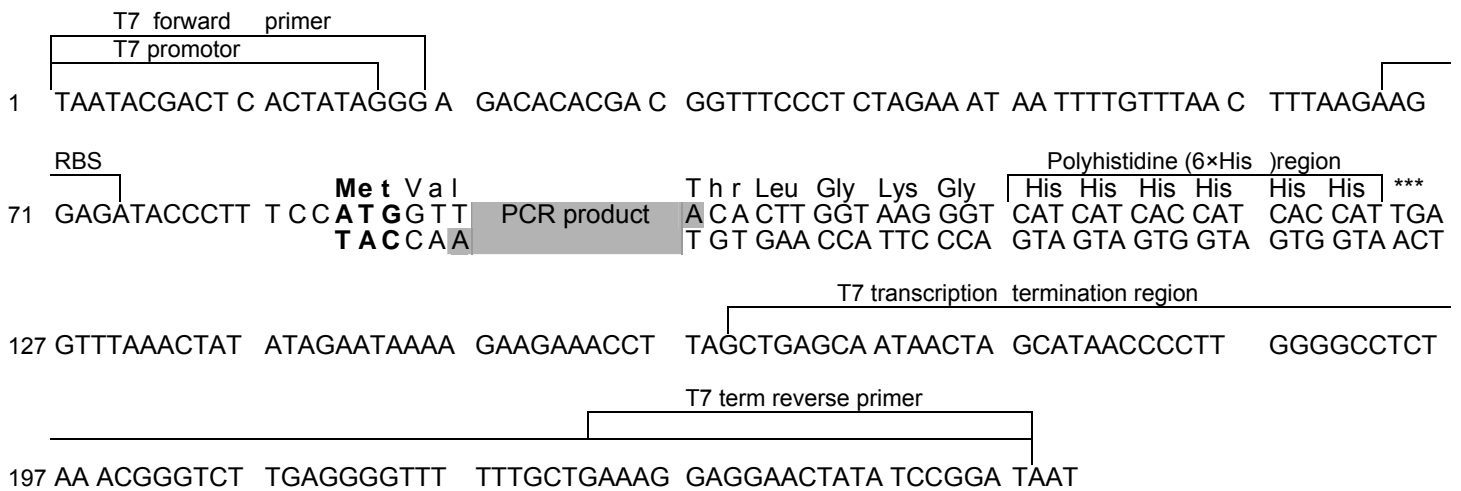
### Long-Term Storage:

Keep the correct clone in 20-30% glycerol and store at -80°C.

### Protein Expression Test:

1. Transform pM-CT TA expression construct into E. coli BL21(DE3), or BL21(DE3)pLysS
2. Select 1 colony and culture in 1ml 2YT medium containing 20 µg/ml ampicillin, pick 4 colonies. Shaking at 37°C until the OD600 reaches 0.4-0.6.
3. Add 1µl 0.5M IPTG into 1ml 2YT culture, Keep 1 colony without IPTG.
4. Shaking at 37°C for 3 hours
5. Pour the culture into 1.5ml eppendorf, centrifuge at maximum speed for 1 minute, and discard supernatant.
6. Resuspend each cell pellet in 100 µl of 1X SDS-PAGE sample buffer.
7. Boil 5 minutes and centrifuge briefly.
8. Load 5-10 µl of each sample on a polyacrylamide gel and electrophorese. Save your samples by storing them at -20°C.
9. Gel staining with coomassie dye

### pM-CT TA vector cloning site:



## **The phenotypes of protein toxicity in molecular biology studies**

- Toxic protein cloning problems
  - No colony in cloning
  - Fewer colonies than those in regular cloning experiments
  - Small percentage of positive clones
  - Wrong orientation
  - Mutations leading to defective products or no expression
- Toxic protein expression problems
  - No expression
  - Low yield
  - Defective proteins
  - The expression seems inconsistent or unpredictable.
- Toxic protein host cell growth rate and cell density problems
  - Cell grows significantly slower to reach its normal density before or after induction.
- Transformation efficiency problems
  - The transformation efficiency of the vector containing a toxic protein is lower than the control