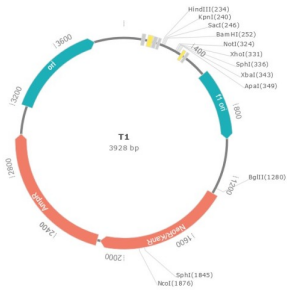
T1 Cloning Kit

Catalogue No.:abx098055



Plasmid map (T1).

T1 Cloning Kit is designed for cloning and sequencing Taq amplified PCR products. 5 minutes fast ligation of Taq-amplified PCR products. T7 promoter primer, M13 forward primer and M13 reverse primer for sequencing. T7 promoter for in vitro transcription.

Kit contents:

Component	**20 rxns**	**60 rxns**
T1 Cloning Vector (10 ng/µl)	20 µl	3 × 20 µl
Control Template (5 ng/µl)	5 µl	5 µl
Control Primer (10 µM)	5 µl	5 µl
M13 Forward Primer (10 µM)	50 µl	150 µl
M13 Reverse Primer (10 µM)	50 µl	150 µl
T1 Phage Resistant Chemically Competent Cells (abx098070)	10 × 100 µl	30 × 100 µl

Target: T1 Cloning Kit

Storage: Store Phage Resistant Chemically Competent Cells at -70°C for up to 6 months and all other components at -20°C for up to 9 months.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.
This product is shipped with dry ice.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

Directions for use:

Preparation:

1. Add 0.5 µl - 4 µl of PCR products and 1 µl of T1 Cloning Vector into a microcentrifuge tube.
2. Gently mix, incubate at room temperature for 5 minutes, and place the tube on ice.

The optimal molar ratio of the vector to insert is 1:7 (for example, 1 kb = 20 ng, 2 kb = 40 ng). The optimal vector volume is 1 µl. The total reaction volume should be 3 µl - 5 µl. The optimal incubation temperature is 25 °C for most inserts, and may be higher (up to 37 °C) for others. The incubation time should be optimised by the end user, and the following recommendations may be used as a guide:

- 0.1-1 kb: 5-10 mins
- 1-2 kb: 10-15 mins
- 2-3 kb: 15-20 mins
- ≥3 kb: 20-30 mins.

Note: If the insert is gel purified, use the maximum incubation time of 20-30 minutes.

Transformation

1. Add the ligated products to 50 µl of T1 Phage Resistant Chemically Competent Cells and mix gently. Do not mix by pipetting up and down.
2. Place on ice for 20-30 minutes.
3. Heat shock the cells at 42°C for 30 seconds, then immediately place the tube on ice for 2 minutes.
4. Add 250 µl of room temperature SOC or LB Medium, then incubate in a shaking incubator (200 rpm) at 37°C for 1 hour.
5. Mix 8 µl of 500 mM IPTG and 40 µl of 20 mg/ml X-gal, and spread evenly on a selective LB plate. Incubate at 37 °C for 30 minutes.
6. Inoculate a pre-warmed culture plate with 200 µl of the transformants.

Identification of positive clones

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

Number of Cycles	Temperature	Time per Cycle
1 cycle	94 °C	10 min
30 cycles	94 °C	30 seconds
	55 °C	30 seconds
	72 °C	Dependent on insert size and PCR Enzyme
1 cycle	72 °C	5-10 min

Note: PCR product size from vector self-ligation is 199 bp.

Sequencing analysis

Positive clones should be inoculated in Ampicillin or Kanamycin selective LB liquid media, then incubate in a shaking incubator (200 rpm) at 37°C for 6 hours. Isolate plasmid DNA for restriction enzyme digestion and DNA sequencing. Sequencing should be performed using M13 forward, M13 Reverse and T7 promoter.

PCR of control insert (700 bp)

Ligate 1 µl of control insert with 1 µl vector, and carry out PCR according to the following conditions:

Required Components

Component	Volume	Final concentration
Control template (5 ng/µl)	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2X PCR SuperMix	25 µl	1X
Nuclease-free water	Variable	-
Total volume	50 µl	-

PCR Conditions

Number of Cycles	Temperature	Time per Cycle
1 cycle	94 °C	2-5 min
30 cycles	94 °C	30 seconds
	50-60 °C	30 seconds
	72 °C	1 min
1 cycle	72 °C	10 min

Notes

- The PCR Enzyme should be a *Taq* DNA Polymerase.
- Primers must not be phosphorylated.
- 5-10 min post extension step is required. After amplification, agarose gel electrophoresis is recommended to validate the quality and quantity of the PCR products.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com