

CYCV15-CHANGYU pTOPO-TA Simple Kit

Contents	20 apps (CYCV1501)	80 apps (CYCV1502)
pTOPO-T Simple Vector(30ng/μl)	20 μl	80 μl
1000bp Control (30ng/μl)	5 μl	5 μl
10 × Enhancer	20 μl	80 μl

Note: All reagents, when store in -20 °C, are stable for 12 months.

❖ Description

The Zero Background TA Simple Topoisomerase Cloning Kit is designed for fast cloning of DNA fragments up to 10 kb generated by Taq DNA polymerase which introduce single 3'-A overhang to the end of the DNA product. DNA fragments generated by restriction digestion or obtained by mechanical shearing can also be effectively cloned after end polishing using Taq DNA polymerase. It utilizes DNA strand transfer activity of Vaccinia virus topoisomerase I. Vaccinia virus DNA topoisomerase I forms a 3'-phosphoryl intermediate with the plasmid vector containing cleavage recognition motif of 5'CCCTT ↓. Covalently bound topoisomerase I then transfer the incised vector DNA strand to the DNA fragment to be cloned with free 5'-OH terminuses. This transferring reaction is rapid and reproducible. The cloning vector pTOPO-TA Simple included in this kit are high copy number plasmids engineered to tolerate mild toxic genes. Regions flanking the cloning site of pTOPO-TA Simple vectors do not contain any common restriction sites which eliminates possible redundancy of restriction sites.

❖ Procedure

1. Set up the topoisomerase cloning reaction by mixing the reagents in the order shown.

DNA Fragment or 1μl 1000bp control	0.5-8μl
pTOPO-T Simple Vector	1μl
10 × Enhancer	1μl
diH2O	Xμl
Final Volume	10μl

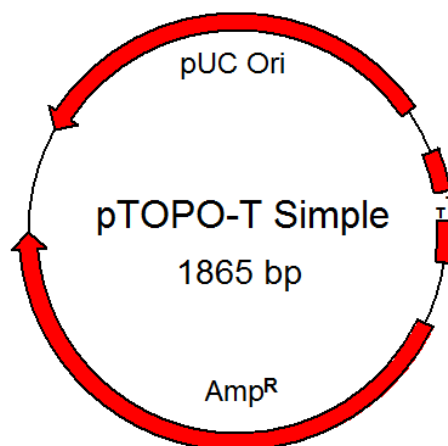
DNA fragments MUST NOT be 5' phosphorylated. TA ended DNA fragment is preferred. Highest cloning efficiency was observed from PCR fragments generated by Taq DNA polymerase or fragments tailored by Taq polymerase. 10-150 ng of

DNA fragments ranging from 100 bp to 5000 bp has been tested to give satisfactory results. Excess amount of DNA inserts e.g. >300ng, will reduce cloning efficiency. Refer to the following table.

Fragment size (bp)	Optimal amount (ng)
100-1000	10-40
1000-2000	40-80
2000-5000	80-150

2. Mix the reaction gently and incubate for 5 minutes at room temperatures between 15-30°C. Recent R&D data indicate incubation at this step produce no detectable benefits. In any case, do not let the incubation go beyond 5 minutes. Extended incubation for larger inserts up to 5 kb is unnecessary and may introduce background.
3. Add 5 μ l of the cloning reaction into 50 μ l chemically competent E. coli and mix gently. Do not mix by pipetting up and down.
4. Incubate on ice for 2-30 minutes.
5. Following the instruction of competent cells to complete transformation.

❖ **Map of pTOPO-T Simple :**



❖ **Sequencing Primer :**

M13F: TGTAACGACGGCCAGT

M13R: CAGGAAACAGCTATGACC

❖ **Cloning site information:**

