

2xHigh-Taq PCR Premix

9K-002-0011 (5x1 ml)

store at -20°C



Protocol

2X High-Taq PCR Premix Solution is a mix composed of High-Taq DNA Polymerase and all required components for easy and simple applications of PCR. PCR reaction can be performed just by adding Template and Primers.

Reaction mixture (for 20µl reaction)		Temperature cycle	
template 1 (10pmole/µl)	10.0µL	95°C 2 min	x1
primer 1 (10pmole/µl)	1.0µL	95°C 20 sec	x20~40
primer 2 (10pmole/µl)	1.0µL	AT* 40 sec	
template	X µL	72°C 1 min/kb	
D.W. to	20.0µL	72°C 5 min	x1

AT*: Annealing Temperature

Adjust to the lower T_m between two primers

$$AT = T_m - (4 \sim 6^\circ\text{C}) \quad T_m = 2^\circ\text{C} \times (A+T) + 4^\circ\text{C} \times (G+C)$$

*It might be required to optimize PCR conditions depending on target size, T_m of primers, template nature, extension time, annealing temperature, enzyme quantity and cycles numbers.

Technical Information

A. Template (temperature cycle)

- . Animal genomic DNA
 - 50-200 ng (25-35 cycles)
 - 10-50 ng (30-40 cycles)
- . Bacterial genomic DNA
 - 10-50 ng (20-25 cycles)
 - 1-5 ng (30-35 cycles)
- . Plasmid and lamda DNA
 - 1-5 ng (20-30 cycles)

B. Primer design

- Primer can be designed using a primer design software or manually.
- Avoid repeated sequence at 3' end.
- In case 3'-end is G+C rich, the end have to be A or T.
- In case 3' end is A+T rich, the end have to be G or C.
- It is recommended that T_m of the designed primers is >64°C and AT >58°C.

C. Extension time

- In general, extension should be performed at 0.5~1.0min/kb
- If the amplification size is more than 3 kb, extension should be performed at 1.0~2.0 min/kb
- If the amplification size is more than 5 kb, extension temperature should be assigned at 68°C.