Recombinant *Taq* DNA Polymerase

TaKaRa Taq™

Code No. R500A **Size : 250 U**

Supplied Reagents :

10×PCR Buffer (Mg^{2+} plus)	1.0 ml

Storage Buffer :

Tris-HCl (pH8.0)	20 mM
KCl	100 mM
EDTA	0.1 mM
DTT	1 mM
Tween 20	0.5%
Nonidet P-40	0.5%
Glycerol	50%

Storage : -20℃

Source :
Escherichia coli carrying a plasmid that encodes the *Thermus aquaticus* DNA Polymerase gene.

Unit definition :
One unit is the amount of enzyme that will incorporate10 nmol of dNTP into acid-insoluble products in 30 minutes at 74℃ with activated salmon sperm DNA as the template-primer.

Reaction mixture for unit definition :

25 mM	TAPS (pH 9.3, 25℃)
50 mM	KCl
2 mM	$MgCl_2$
0.1 mM	DTT
200 µM	each dATP·dGTP·dCTP
100 µM	[^{3}H]-dTTP
0.25 mg/ml	activated salmon sperm DNA

Purity :
Nicking, endonuclease and exonuclease activity were not detected after the incubation of 0.6 µg of supercoiled pBR322 DNA, 0.6 µg of λDNA or 0.6 µg of λ-*Hin* d III digest with 10 units of this enzyme for 1 hour at 74℃.

Applications :
1) For DNA amplification by PCR
2) For DNA sequencing

PCR products :
As most PCR products amplified with *TaKaRa Taq* have one A added at the 3'-termini, the obtained PCR product can be directly cloned into a T-vector. Also it is possible to clone the product in a blunt-end vector after blunting and phosphorylation of the ends.

PCR test :
Good performance was confirmed by PCR amplification of an 8 kb fragment using λDNA template.

General reaction mixture for PCR (50 µl reaction volume) :

TaKaRa Taq (5 U/µl)	0.25 µl
10×PCR Buffer (Mg^{2+} plus)	5 µl
dNTP Mixture (各 2.5 mM)	4 µl
Template	<500 ng
Primer 1	0.2 - 1.0 µM (final conc.)
Primer 2	0.2 - 1.0 µM (final conc.)
Sterile purified water	up to 50 µl

PCR conditions (an example) :
Amplification of a 1 kb DNA fragment

98℃	10 sec	⎫
55℃	30 sec	⎬ 30 Cycles
72℃	1 min	⎭

(Note) Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. The recommendation is 5 - 10 sec at 98℃ or 20 - 30 sec at 94℃.

Supplied 10x PCR Buffer (Mg^{2+} plus)

Tris-HCl (pH8.9)	100 mM
KCl	500 mM
$MgCl_2$	15 mM

< Cool Start Method >
The "Cool Start Method" provides more accurate amplification and minimizes nonspecific amplification. This is a simple method that does not require specialized enzymes or additional reagents.

Protocol of Cool Start Method
1、 Keep all reagents on ice until use.
2、 Prepare the reaction mixture on ice.*1, 2
*1 : Order of reagent addition does not influence results.
*2 : Results will not be affected by leaving the mixture on ice for 30 min before thermal cycling.
3、 Set a thermal cycler with the designated program.*3
*3 :PCR conditions do not need to be changed for Cool Start.
4、 Set the tubes in a thermal cycler and start thermal cycling immediately.