TaKaRa LA Taq®
DNA Polymerase

Code No. RR002M
Size: 250 units

Shipping at − 20℃
Store at − 20℃

Supplied Reagents :

10X LA PCR Buffer II (25 mM Mg^{2+} plus)	**1 ml**
dNTP Mixture (2.5 mM each)	**800 μl**

Lot No.
Conc. : **units/μl**
Volume : **μl**
Expiration Date :

Storage Buffer :

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

Supplied dNTP Mixture (2.5 mM each)
The dNTP Mixture is ready for use in PCR without dilution.
Form : Dissolved in water (sodium salts), pH7 - 9
Purity : ≧ 98% for each dNTP

Unit definition :
One unit is the amount of enzyme that incorporates10 nmol of dNTPs 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 (pH9.3 at 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 incubation of 0.6 μg of supercoiled pBR322 DNA, 0.6 μg of λ DNA, or 0.6 μg of λ-*Hin*d III digest with 10 units of this enzyme for 1 hour at 74℃.

Applications : For DNA amplification by PCR. This enzyme is optimized for long range PCR (>15 kb fragments).

PCR products :
As most PCR products amplified with *TaKaRa LA Taq* DNA polymerase have one A at the 3'-termini, the obtained PCR products can be directly used for cloning into T-Vectors. When cloning long products (>5 kb) into T-Vectors, the cloning efficiency may be low. It is also possible to clone the product in blunt-end vectors after blunting and phosphorylation of the end.

PCR test :
Good performance in PCR was confirmed by amplification of a 35 kb fragment from λ DNA template.
Good performance in PCR was confirmed by amplification of the β-globin gene (17.5 kb) using human genomic DNA template.

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

TaKaRa LA Taq (5 units/μl)	0.5 μl
10X LA PCR Buffer II (Mg^{2+} plus)	5 μl
dNTP Mixture (2.5 mM each)	8 μl
Template	<1 μg
Primer 1	0.2 - 1.0 μM (final conc.)
Primer 2	0.2 - 1.0 μM (final conc.)
Sterile distilled water	up to 50 μl

PCR conditions (example) : Amplification of a 17.5 kb DNA fragment

94℃	1 min.	
98℃	10 sec.	] 30 cycles
68℃	15 min.	
72℃	10 min.	

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

< Cool Start Method >
The "Cool Start Method" provides more accurate amplification and minimizes amplification of nonspecific products. This simple method does not require specialized enzymes or additional reagents.
Cool Start Method Protocol
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 up to 30 min. before thermal cycling.
3) Set the thermal cycler with the designated program.＊3
　＊3 : PCR conditions do not need to be changed for the Cool Start Method.
4) Set the tubes in the thermal cycler and start cycling immediately.