

TaKaRa LA Taq® DNA Polymerase

Code No. RR002M
Size: 250 units

Shipping at - 20°C
Store at - 20°C

Supplied Reagents :

10X LA PCR Buffer II (25 mM Mg²⁺ 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 incorporates 10 nmol of dNTPs into acid-insoluble products in 30 minutes at 74°C with activated salmon sperm DNA as the template-primer.

Reaction mixture for unit definition :

25 mM	TAPS (pH9.3 at 25°C)
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-Hind III digest with 10 units of this enzyme for 1 hour at 74°C.

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°C	1 min.	} 30 cycles
98°C	10 sec.	
68°C	15 min.	
72°C	10 min.	

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

NOTICE TO PURCHASER: LIMITED LICENSE

[P1] PCR Notice

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[M57] LA Technology

This product is covered by the claims 6-16 outside the U.S. corresponding to the expired U.S. Patent No. 5,436,149.

TaKaRa LA Taq is a registered trademark of TAKARA BIO INC.

Note

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