

TaKaRa LA Taq™

Code No. HRR002A
Size: 125 units

Shipping at — 20°C
Store at — 20°C

Supplied	10X LA PCR™ Buffer II (Mg ²⁺ free)	1 ml
Reagents :	25 mM MgCl ₂	1 ml
	dNTP Mixture	400 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%	NP-40
	50%	Glycerol

Supplied dNTP Mixture

dNTP Mixture is ready for use in PCR without dilution.

Concentration	: 2.5 mM of each dNTP
Form	: Dissolved in water (sodium salts), pH7 - 9
Purity	: ≥ 98% for each dNTP

Unit definition : One unit is the amount of enzyme that will incorporate 10 nmol of dNTP 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 the 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 Polymerase Chain Reaction (PCR).

PCR products : As most PCR products amplified with TaKaRa LA Taq™ have one A added at 3'-termini, the obtained PCR product can be directly used for cloning into T-Vector. But in the case of cloning the long length product (>5 kb) into T-Vector, the cloning efficiency becomes quite low. Also it is possible to clone the product in blunt-end vectors after blunting and phosphorylation of the end.

PCR test : Good performance of DNA amplification by PCR was confirmed by using λ DNA as the template (amplified fragment : 35 kb). Good performance of DNA amplification of β-globin gene (17.5 kb) by PCR was also confirmed by using human genome DNA as the template.

General reaction mixture for PCR (total 50 µl)

TaKaRa LA Taq™ (5 units/µl)	0.5 µl
10X LA PCR™ Buffer II (Mg ²⁺ free)	5 µl
25 mM MgCl ₂ (final 2.5 mM)	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.)
Sterilized distilled water	up to 50 µl

PCR condition (an example) : when amplifying 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. The recommendation is for 1 - 10 sec. at 98°C or 10 - 30 sec. at 94°C.

< Cool Start Method >

"Cool Start Method" + provides more accurate amplification and minimizes amplification of nonspecific bands. This is a simple method that does not require specialized enzymes or additional reagents. Higher reaction specificity can be achieved by combining Hot Start PCR techniques with Taq Antibody (Cat. #9002A) and Cool Start method.

Protocol of Cool Start Method

- 1) Keep all reagents on ice until use.
 - 2) Prepare the reaction mixture on ice.
 - * 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 dose not need to be changed for Cool Start.
 - 4) Set the tubes in a thermal cycler and start thermal cycling immediately.
- + : JAPAN Patent 2576741 for Cool Start Method is owned by SHIMADZU CORPORATION

NOTICE TO PURCHASER: LIMITED LICENSE

[P1] PCR Notice

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[M57] LA Technology

This product is covered by the claims 6-16 of U.S. Patent No. 5,436,149 and its foreign counterpart patent claims.

Note

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