TaKaRa LA Taq™

Code No. HRR002A
Size: 125 units

Shipping at − 20℃
Store at − 20℃

Supplied Reagents :	**10X LA PCR™ Buffer II (Mg^{2+} free)**	**1 ml**
	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 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 (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 the 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 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 genone 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℃ 1 min.
98℃ 10 sec.
68℃ 15 min.] 30 cycles
72℃ 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℃ or 10 - 30 sec. at 94℃.

< 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,*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 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

Produced by TAKARA BIOTECHNOLOGY (DALIAN) CO., LTD.

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