TaKaRa Ex Taq™

Code No. HRR001A
Size: 250 units

Shipping at − 20℃
Store at − 20℃

Supplied Reagents : **10X *Ex Taq* Buffer** **1 ml**
dNTP Mixture **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%	NP-40
50%	Glycerol

Supplied 10X *Ex Taq* Buffer : Mg^{2+} concentration (10X) : 20 mM

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), pH 7 - 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 (pH 9.3 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 Ex Taq*™ have one A added at 3'-termini, the obtained PCR product can be directly used for cloning into T-vector. 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 : 20 kb).
Good performance of DNA amplification of β-globin gene by PCR was also confirmed by using human genomic DNA as the template (amplified fragment : 17.5 kb).

General reaction mixture for PCR (total 50 μl)

TaKaRa Ex Taq™ (5 units/μl)	0.25 μl
10X *Ex Taq* Buffer	5 μl
dNTP Mixture (2.5 mM each)	4 μl
Template	< 500 ng
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 1 kb DNA fragment

98℃ 10 sec.
55℃ 30 sec.] 30 cycles
72℃ 1 min.

or

98℃ 10 sec.
68℃ 1 min.] 30 cycles

(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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