10XPCR Buffer,dNTP Mixture for PCR

TaKaRa Ex Taq™

TaKaRa

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Code No. RR001A
Size: 250 units

Shipping at -20°C
Stored at -20°C

Supplied Reagents : 10X *Ex Taq*™ Buffer
dNTP Mixture

Lot No. E9901-3
Concentration : 5 units/μl
Volume : 50 μl
Expiry Date : Dec. 2004

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

Unit definition: One unit is the amount of the enzyme that will incorporate10 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 (pH 9.3 at 25°C)
50 mM	KCl
2 mM	$MgCl_2$
1 mM	2-mercaptoethanol
200 μM	each dATP,dGTP,dTTP
100 μM	[α-^{32}P]-dCTP
0.25 mg/ml	activated salmon sperm DNA

Purity: Nicking activity, 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°C.

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 Polymerase Chain Reaction (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).

PCR condition (an example)
When amplifying 1 kbp DNA fragment

98°C	10 sec	30 cycles	or	98 °C	10 sec	30 cycles
55°C	30 sec			68 °C	1 min	
72°C	1 min					

Note:Denaturation condition varies depending on an used thermal cycler and tube. It is recommended for 10-30 sec. at 94°C, or 1-10 sec. at 98°C.

General reaction mixture for PCR (total 100 μl)

TaKaRa Ex Taq ™ (5 units/μl)	0.5 μl
10X *Ex Taq* ™ Buffer	10 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 100 μl

Supplied 10X *Ex Taq* ™ Buffer

Supplied Size	: 1 ml/vial
Mg^{2+} concentration (10X)	: 20 mM
Storage	: –20°C

Supplied dNTP Mixture
Mixture of dNTP, ready for use in Polymerase Chain Reaction (PCR) without dilution.

Supplied Size	: 800 μl/vial
Concentration	: 2.5 mM of each dNTP
pH	: pH 7 ~ 9
Form	: Solved in water (sodium salts)
Purity	: ≥ 98% for each dNTP
Storage	: –20°C

< Cool Start Method >
"Cool Start Method' , enables to minimize the amplification of non-specific band in PCR and achieves more accurate amplification. This is a simpler method without need for special enzyme nor additional reagents.⁺ Higher reaction specificity can be achieved by combining Hot Start PCR techniques with Taq Antibody (Code.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.*,**
   *The adding order of reagents dose not influence on results.
   **The result will not be affected even when the mixture is left on ice 30 min. before thermal cycling.
3) Set a thermal cycler ready to start with the designated program.***
   ***No need to change PCR conditions especially 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

Note
For research use only. Not for use in diagnostic or therapeutic procedures.

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