Cat. # R006A

For Research Use

TaKaRa

TaKaRa Z-Taq™ DNA Polymerase

Product Manual

v201411Da

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I. Description

TaKaRa Z-Taq is a modified *Taq* polymerase designed for rapid PCR. *TaKaRa Z-Taq* is a highly robust enzyme that has a five-fold higher processing speed compared to conventional *Taq* polymerase, allowing amplification of 1 kb of DNA in just 20 min. Because of this excellent processivity, *TaKaRa Z-Taq* can be used to amplify large portions of human genomic, lambda, or bacterial DNA. Furthermore, the enzyme is very robust, allowing the number of PCR cycles to be increased up to 50; these additional cycles improve the detection sensitivity for reactions with low amounts of DNA template.

Due to the robust activity and high processing speed of *TaKaRa Z-Taq*, it is well-suited for high-throughput applications.

II. Components

TaKaRa Z-Taq (2.5 units/μl)	200 U
10X *Z-Taq* Buffer (Mg^{2+} plus, 30 mM)	800 μl
dNTP Mixture (2.5 mM each)	800 μl

【*TaKaRa Z-Taq* 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

III. Specifications

・Reaction speed:	5-fold faster than *Taq* polymerase
・Thermostability:	Half-life at 92.5℃ is >130 min.
・Amplification length:	
E. coli genomic DNA	<20 kb
Human genomic DNA	<17.5 kb
λ DNA	<20 kb
・Reverse transcriptase activity:	equivalent to *Taq* polymerase
・Associated exonuclease activity:	3' → 5' +
	5' → 3' ±
・Terminal of amplified fragment:	A-overhang at 3'-termini
・Optimal reaction temperature:	approximately 70℃

IV. Optimization of Reaction Conditions

For best results, optimize each PCR parameter fully.

IV-1. PCR cycling conditions (Reaction volume: 50 μl)

◆ Shuttle PCR

98℃ 1 - 5 sec.
68℃ X sec.*] 25 - 30 cycles

＊: The optimal annealing/extension time varies depending on the size of the amplified fragment. The recommended time is 10 - 20 sec./kb.

Examples (Shuttle PCR):

Amplification of 2 kb

98℃ 1 (or 5) sec.
68℃ 20 sec.] 30 cycles

Amplification of 8 kb

98℃ 1 (or 5) sec.
68℃ 80 sec.] 30 cycles

Amplification of 20 kb

98℃ 1 (or 5) sec.
68℃ 200 sec.] 30 cycles

◆ 3-step PCR can be performed by setting the extension time to 1/3 - 1/5 of the time required for *Taq* DNA polymerase.

Examples (3-step PCR):

Amplification of 2 kb

98℃ 1 (or 5) sec.
55℃ 1 - 10 sec.
72℃ 10 - 20 sec.] 30 cycles

Amplification of 8 kb

98℃ 1 (or 5) sec.
55℃ 1 - 10 sec.
72℃ 80 sec.] 30 cycles

Amplification of 20 kb

98℃ 1 (or 5) sec.
55℃ 1 - 10 sec.
72℃ 200 sec.] 30 cycles

Note :

1) Longer reaction times may cause diffuse, smeared electrophoresis bands for both Shuttle PCR and 3-step PCR.
2) When performing Shuttle PCR, poor results might occur depending on primer sequence or template length. In that case, perform 3-step PCR.

VI-2. Template DNA

Optimal amount of template DNA:

Human genomic DNA	50 - 500 ng/50 μl reaction
E. coli genomic DNA	1 - 100 ng/50 μl reaction
λ DNA	50 pg - 5 ng/50 μl reaction
Plasmid DNA	0.5 - 50 pg/50 μl reaction

The optimal amount of template DNA may vary depending on the purity of the DNA preparation.

VI-3. Primers

Optimal concentration	0.2 - 1 μM
Optimal primer length	20 - 30 mers

Primer	Recommended for amplification
5' FITC labeled	Yes
5' ROX labeled	Yes
5' biotin labeled	Yes
containing dUTP	Yes
containing dITP	No

VI-4. dNTP and Mg^{2+}

Because dNTPs chelate Mg^{2+}, a high concentration of dNTPs lowers the effective concentration of Mg^{2+}.

Optimal Mg^{2+} concentration	3 mM (final conc.)
Optimal concentration of dNTPs	200 μM each (final conc.)

Excess Mg^{2+} leads to non-specific amplification, and too little Mg^{2+} leads to low reactivity. The presence of chelating agents (e.g., EDTA) decreases the effective concentration of Mg^{2+}. The concentration of Mg^{2+} should be higher than the total concentration of dNTPs.
The concentration of each individual dNTP should be the same. If it is not, misincorporation will occur.

VI-5. Incorporation of Nucleoside Triphosphate Analogs

Substrate	Amplification
dUTP	−
Biotin-16-dUTP	−
Biotin-14-dATP	−
7-deaza-dATP	+
7-deaza-dGTP	+
Methyl-dCTP	−
DIG-dUTP	−

Substrate	Amplification
dUTP：dTTP = 1：1	−
Biotin-16-dUTP：dTTP = 1：1	−
Biotin-14-dATP：dATP = 1：1	+
7-deaza-dATP：dATP = 1：1	+
7-deaza-dGTP：dGTP = 1：1	+
Methyl-dCTP：dCTP = 1：1	+
DIG-dUTP：dTTP = 1：1	−

VI-6. Reaction Volume

The recommended volume of the reaction mixture is 50 μl.
Denaturation temperature and time should be re-optimized if the reaction volume is changed.

V. Preparation of reaction mixture

Keep reagents on ice after thawing. To prevent non-specific amplification due to misannealing of primers, keep the PCR mixture on ice after preparation.
Add each reagent in the following order, then mix gently by pipetting.

Order of addition

1) H_2O
2) 10X *Z-Taq* Buffer
3) dNTP Mixture
4) Template DNA
5) *TaKaRa Z-Taq*
6) Primer 1
7) Primer 2

Start PCR promptly after the mixture is prepared.

∗ For multiple reactions, prepare a master mix consisting of *TaKaRa Z-Taq*, 10X *Z-Taq* Buffer, and dNTP Mixture.

PCR composition (50 μl volume)

TaKaRa Z-Taq (2.5 units/μl)	0.5 μl	
10X *Z-Taq* Buffer	5 μl	
dNTP Mixture (2.5 mM each)	4 μl	(final conc. 200 μM)
Template DNA	<1 μg	
Primer 1	10 pmol	(final conc. 0.2 μM)
Primer 2	10 pmol	(final conc. 0.2 μM)
Sterile distilled water	to 50 μl	

VI. Effect of Additives

A 2 kb target was amplified from *E. coli* genomic DNA (100 pg/50 μl reaction).

Cycling conditions:

98℃ 5 sec.
66℃ 20 sec.] 35 cycles

The effect of each additive was examined by comparing the amount of product amplified in the presence of the indicated concentration of additive. The control reaction contained no additives.

Additives	Final conc.	Effect on PCR
Formamide	1%	No inhibition
	5%	Complete inhibition
	10%	Complete inhibition
DMSO	1%	No inhibition
	5%	50% inhibition
	10%	Complete inhibition
Nonidet P-40	0.01%	80 - 90% inhibition
	0.1%	80 - 90% inhibition
Tween 20	0.1%	No inhibition
	1%	No inhibition
Triton X-100	0.1%	No inhibition
	1%	Complete inhibition
SDS	0.01%	Complete inhibition
	0.1%	Complete inhibition
Glycerol	1%	No inhibition
	5%	90% inhibition
PEG 6000	0.1%	No inhibition
	1%	No inhibition
EDTA	0.5 mM	No inhibition
	5 mM	Complete inhibition
LB	1%	No inhibition
	10%	90% inhibition
DTT	1 mM	No inhibition
	10 mM	No inhibition
Gelatin	0.01%	No inhibition
	0.1%	Smeared bands may occur
BSA	0.01%	No inhibition
	0.1%	No inhibition

VII. Troubleshooting

Little or no amplification

Possible Causes	*Suggestions*
Extension time is too short.	Set extension step conditions as to 10 - 20 sec./kb at 68 - 72℃.
Annealing temperature is too high.	Decrease temperature by 2℃ decrements, or perform touchdown PCR.
Annealing time is too short.	Set annealing time as 30 sec. to 1 min.
GC content of template may be high, or the template may have complex secondary structure.	Increase denaturation temperature or time to 98℃ for 1 - 10 sec. or 94℃ for 10 - 30 sec.
Primers are not suitable.	・ Increase primer purity. ・ Adjust GC content of primer to ~50%. ・ Use a longer primer (20 - 30 mer). ・ Ensure that primers do not have complementarity at their 3' - ends.
Primer concentration is too low.	Use primers at a final concentration of 0.2 - 1 μM.
Denaturing conditions are inappropriate.	Set the denaturation step conditions as 98℃ for 1 - 10 sec. or 94℃ for 10 - 30 sec.
Template DNA is not pure enough.	Re-purify template DNA.
Insufficient amount of template DNA.	Use an appropriate amount of template. Human genomic DNA ~ 500 ng/50 μl PCR *E. coli* genomic DNA ~ 100 ng/50 μl PCR λ DNA ~ 5 ng/50 μl PCR Plasmid DNA ~ 50 pg/50 μl PCR
Insufficient number of cycles.	Perform ~50 cycles of PCR.

Extra bands

Possible Causes	*Suggestions*
Annealing temperature is too low.	・Raise the temperature in increments of 2℃. ・Perform touchdown PCR.
Excessive template DNA.	Use an appropriate amount of template. Human genomic DNA ~ 500 ng/50 μl PCR *E. coli* genomic DNA ~ 100 ng/50 μl PCR λ DNA ~ 5 ng/50 μl PCR Plasmid DNA ~ 50 pg/50 μl PCR
Excessive number of PCR cycles.	・ Perform <50 PCR cycles. ・ Reduce the number of cycles at 2 cycle intervals.
Excessive primer.	Use primers at 0.2 - 1 μM (final conc.).
Primers are too long.	Design primers that are >30 mer.

Smeared bands

Possible Causes	*Suggestions*
Extension time is too long.	Set to 10 - 20 sec./kb at 68 - 72℃.
Excessive number of PCR cycles.	Perform <50 PCR cycles.
Excessive template DNA.	Use an appropriate amount of template. Human genomic DNA ~ 500 ng/50 μl PCR *E. coli* genomic DNA ~ 100 ng/50 μl PCR λ DNA ~ 5 ng/50 μl PCR Plasmid DNA ~ 50 pg/50 μl PCR

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