Cat. # R050A

For Research Use

TaKaRa

PrimeSTAR® GXL DNA Polymerase

Product Manual

v201509Da

Table of Contents

I. Description

PrimeSTAR GXL DNA Polymerase is a revolutionary PCR enzyme that augments the high-fidelity PrimeSTAR HS DNA Polymerase with a novel elongation factor to dramatically increase PCR performance.

The superior performance of PrimeSTAR GXL DNA Polymerase is unsurpassed by other commercially available high-fidelity PCR enzymes. PrimeSTAR GXL DNA Polymerase allows amplification of products ≧30 kb in length, while maintaining exceptionally high fidelity. Suitable for GC-rich templates that are otherwise difficult to amplify, this enzyme enables successful amplification from challenging templates without the need for extensive optimization of buffers or reaction conditions. In addition, PrimeSTAR GXL DNA Polymerase is compatible with a wide range of template quantities, and is capable of robust amplification even in the presence of large excesses of non-target DNA. Excess non-target nucleic acid typically inhibits the performance of conventional high-fidelity PCR enzymes. With PrimeSTAR GXL DNA Polymerase, detection of cDNA corresponding to rare transcripts is readily achieved.

Furthermore, an antibody-mediated hot-start formulation prevents false initiation events during reaction assembly due to mispriming or primer digestion.

The PCR extension time recommended in the Standard Protocol is 1 min/kb. However, by doubling the quantity of enzyme used, it is also possible to perform rapid PCR on a wide variety of targets using an extension time of 10 sec/kb.

II. Components (200 reactions)*1

PrimeSTAR GXL DNA Polymerase (1.25 U/μl)*2	200 μl
5X PrimeSTAR GXL Buffer (Mg^{2+} plus)*3	1 ml x 2
dNTP Mixture (2.5 mM each)	800 μl

*1: Assuming a 50 μl reaction volume

*2: 【Storage Buffer】

50 mM	Tris-HCl (pH 8.2 at 4℃)
100 mM	NaCl
0.1 mM	EDTA
1 mM	DTT
0.1%	Tween 20
0.1%	Nonidet P-40
50%	Glycerol

【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℃ with activated salmon sperm DNA as the template.

*3: Mg^{2+} concentration: 5 mM (5X).

III. Storage

−20℃

IV. Protocols

Two protocols are described: a Standard Protocol, in which an extension time of 1 min/kb is used; and a Rapid PCR Protocol, in which extension can be conducted at 10 sec/kb by using twice the quantity of enzyme.
PCR reaction mixtures can be prepared at room temperature. However, keep each of the reaction components on ice while preparing the reaction mixture.

A. Standard Protocol

- Composition of the PCR Reaction Mixture

		Final conc.
5X PrimeSTAR GXL Buffer	10 μl	1X
dNTP Mixture (2.5 mM each)	4 μl	200 μM each
primer 1	10 - 15 pmol	0.2 - 0.3 μM *
primer 2	10 - 15 pmol	0.2 - 0.3 μM *
Template	Refer to Section V.3. Optimization of Parameters	
PrimeSTAR GXL DNA Polymerase	1 μl	1.25 U/50 μl
Sterile distilled water	to 50 μl	

＊: When amplifying products ≧10 kb in length, use primers at a final concentration of 0.2 μM each.

- PCR Conditions

[For ≦10 kb products]

98℃	10 sec	┐
55 or 60℃＊1	15 sec	├ 30 cycles [3-step PCR]
68℃＊2	1 min/kb	┘
- or -		
98℃	10 sec	┐
68℃＊2	1 min/kb	┘ 30 cycles [2-step PCR]

＊1: When the Tm value (calculated by the following formula＊) is greater than 55℃, set the annealing temperature to 60℃. When the Tm value is 55℃ or less, set the annealing temperature to 55℃.

＊: Tm value calculation method:
Tm (℃) = 2(NA + NT) + 4(NC + NG) - 5
where N represents the number of nucleotides in the primer having the specified identity (A, T, C, or G)

＊2: For both 2-step and 3-step PCR, set the extension temperature to 68℃.

[For 10 to 30 kb products]

98℃	10 sec	┐
68℃	10 min	┘ 30 cycles

[For ≧30 kb products]

98℃	10 sec	┐
68℃	15 min	┘ 30 cycles

◆ Selecting PCR Conditions

- For amplification of products ≦10 kb in length, try 3-step PCR first.
- When using GC-rich templates or amplifying products ≧10 kb in length, 2-step PCR is recommended.
- If amplified products are not obtained, or if a smear or a non-specific band(s) is observed during electrophoretic analysis, refer to Section VII. Troubleshooting.

B. Rapid PCR Protocol

- Composition of PCR Reaction Mixture

		Final conc.
5X PrimeSTAR GXL Buffer	10 μl	1X
dNTP Mixture (2.5 mM each)	4 μl	200 μM each
primer 1	10 - 15 pmol	0.2 - 0.3 μM*
primer 2	10 - 15 pmol	0.2 - 0.3 μM*
Template	Refer to Section V.3. Optimization of Parameters	
PrimeSTAR GXL DNA Polymerase	2 μl	2.5 U/50 μl
Sterile distilled water	to 50 μl	

*: When amplifying products ≧10 kb in length, use primers at a final concentration of 0.2 μM each.

- PCR Conditions

[For ≦10 kb products]

98℃	10 sec	] 30 cycles [3-step PCR]
55 or 60℃*1	15 sec	
68℃*2	10 sec/kb	

1: When the Tm value (calculated by the following formula) is more than 55℃, set the annealing temperature to 60℃. When the Tm value is 55℃ or less, set the annealing temperature to 55℃.

*: Tm value calculation method:

$$Tm\ (℃) = 2(NA + NT) + 4(NC + NG) - 5$$

where N represents the number of nucleotides in the primer having the specified identity (A, T, C, or G)

*2: For 3-step PCR, set the extension temperature to 68℃.

[For 10 to 20 kb products]

98℃	10 sec	] 30 cycles [2-step PCR]
68℃	20 sec/kb	
- or -		
98℃	10 sec	] 30 cycles [3-step PCR]
60℃	15 sec	
68℃	10 sec/kb	

◆ Selecting PCR Conditions

- For amplification of products ≦10 kb in length, perform 3-step PCR. 2-step PCR is not recommended for products of this size.
- For amplification of products ≧10 kb in length, 3-step PCR is recommended when a shorter reaction time is desired, and 2-step PCR is recommended when enhanced specificity is desired.
- For GC-rich templates, use the Standard Protocol.
- If amplified products are not obtained, or if a smear or a non-specific band(s) is observed in electrophoretic analysis, refer to Section VII. Troubleshooting.

V. Optimization of Parameters

In order to obtain the best PCR results, it is important to optimize the PrimeSTAR GXL DNA Polymerase reaction parameters to fully utilize the enzyme's properties and advantages.

(1) Primer design

Select primer sequences using primer design software such as OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.).

[For ≦10 kb products]

For general amplification, 20- to 25-mer primers are suitable. Selection of primers with a Tm value of ≧55℃ (as calculated using the formula in Section IV. PCR Conditions) or greater than 25-mer in length may provide optimal results. See Section IV. Protocols.

[For >10 kb products]

Design primers that are 25- to 35-mers and that have a Tm value of ≧65℃.

Avoid high GC-content at the 3' end of each primer.

[For GC-rich amplification products]
Design primers to have Tm values >60℃.

Note:
Do not use inosine-containing primers with PrimeSTAR GXL DNA Polymerase.

(2) dNTP and Mg^{2+}

dNTPs are capable of chelation, and therefore the concentration of free Mg^{2+} in a reaction mix is inversely related to dNTP concentration. The PrimeSTAR GXL Buffer is formulated to result in a final (1X) concentration of 1 mM Mg^{2+} when the final (1X) concentration of dNTPs is 200 μM each. Avoid changing the dNTP concentration as much as possible.

Do not use dUTP with PrimeSTAR GXL DNA Polymerase. dUTP will greatly affect enzyme activity.

(3) Template

Recommended quantities of template DNA (assuming a 50 μl reaction):

	(for general conditions)	(for long PCR products)
Human genomic DNA	5 ng - 500 ng	100 ng - 500 ng
E. coli genomic DNA	100 pg - 200 ng	10 ng - 200 ng
Plasmid DNA	10 pg - 10 ng	1 ng - 10 ng
cDNA	25 ng - 750 ng	250 ng - 750 ng

Do not use templates containing uracil, such as bisulfite-treated DNA.

VI. Electrophoresis, and Cloning of Amplified Products

(1) Electrophoresis

TAE Buffer is recommended for agarose gel electrophoresis of amplified products that are obtained using PrimeSTAR GXL DNA Polymerase.

Note: TBE Buffer may result in DNA band patterns that are enlarged at the bottom of the gel.

(2) Termini of amplified products

Most PCR products amplified with PrimeSTAR GXL DNA Polymerase have blunt-end termini. Therefore, they can be cloned directly into blunt-end vectors. If necessary, phosphorylate amplified products before cloning. Use the Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) for cloning into a blunt-end vector.

(3) Restriction enzyme digestion

Prior to performing restriction enzyme digestion of amplified PCR products, remove all traces of PrimeSTAR GXL DNA Polymerase from the reaction mixture by phenol/chloroform extraction or by using the NucleoSpin Gel and PCR Clean-Up (Cat. #740609.50/.250) kit. For restriction enzymes that produce a 3'-overhang, such as *Pst* I, the 3'-overhang may be removed by the 3'→5' exonuclease activity of PrimeSTAR GXL DNA Polymerase if residual polymerase remains in the restriction digest reaction.

VII. Troubleshooting

Event	Possible Causes	Action
No amplification or poor amplification efficiency	Primer Tm	Refer to Section V. (1) Optimization of Parameters - Primer design
	Annealing temperature	Lower by 2℃ per trial
	Primer concentration	Use in the range of 0.3 to 0.5 μM (final conc.)
	PCR conditions	Try the Rapid PCR Protocol
	Number of cycles	Use 35 to 40 cycles
	Purity and quantity of template DNA	Use an appropriate amount of template DNA Purify the template DNA
Electrophoretic analysis shows a smeared band(s) or extra band(s)	Primer Tm	Refer to Section V. (1) Optimization of Parameters - Primer design
	Annealing temperature	Raise by 2℃ per trial up to to 63℃ Try 2-step PCR For a primer Tm value of 50℃ or less, set in the range of 50 to 55℃
	Extension time	When amplification product is ≦1 kb, set to 10 sec/kb
	Primer concentration	Use at a final concentration of 0.2 μM each
	Number of cycles	Use 25 to 30 cycles
	Template DNA purity	Purify the template DNA

VIII. Features

A. Accuracy

The mutation frequency of PrimeSTAR GXL DNA Polymerase was examined by analysis of sequencing data.

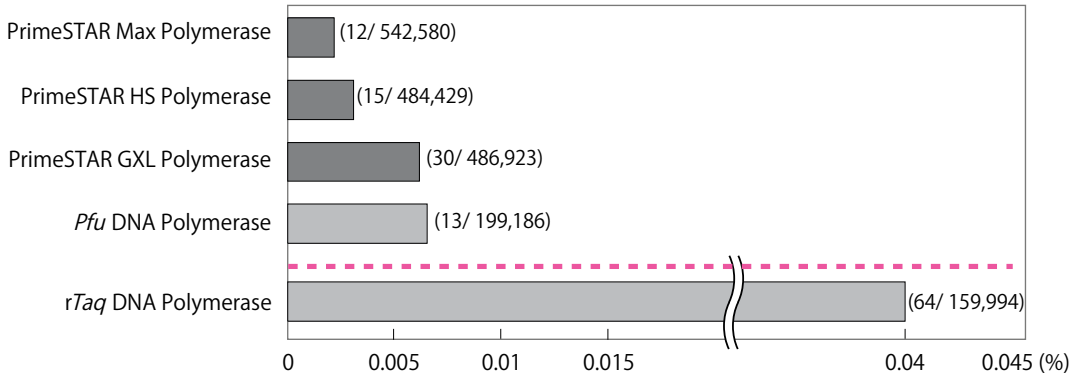
[Method]

Ten arbitrarily selected GC-rich regions were amplified with PrimeSTAR GXL DNA Polymerase or other DNA polymerases using *Thermus thermophilus* HB8 genomic DNA as a template.

Each PCR product (approx. 500 bp each) was cloned into a suitable plasmid. Multiple clones were selected for each amplification product and were subjected to sequence analysis.

[Result]

Sequence analysis of DNA fragments amplified using PrimeSTAR GXL DNA Polymerase showed only 30 mismatched bases per 486,923 total bases. This is higher fidelity than *Pfu* DNA polymerase.

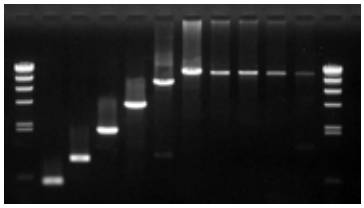


B. Length of amplification products

PrimeSTAR GXL DNA Polymerase enables the amplification of long DNA fragments that cannot be obtained using other commercially available high-fidelity PCR enzymes. As shown below, amplification was confirmed for fragments up to 30 kb in length using human genomic DNA as the template, up to 40 kb in length using λ DNA as the template, and up to 13.5 kb in length using cDNA as the template.

Human genomic DNA
(Template: 100 ng/50 μl reaction)

M 1 2 3 4 5 6 7 8 9 10 M

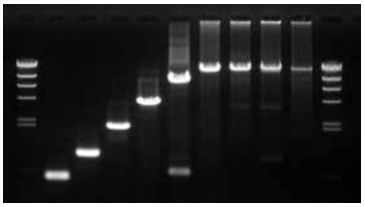


1. p53 0.5 kb
2. DCLRE 1A 1 kb
3. DCLRE 1A 2 kb
4. DCLRE 1A 4 kb
5. β-globin 8.5 kb
6. β-globin 15 kb
7. β-globin 20 kb
8. β-globin 24 kb
9. β-globin 27 kb
10. β-globin 30 kb

M. λ-*Hin*d III digest

λDNA
(Template: 1 ng/50 μl reaction)

M 1 2 3 4 5 6 7 8 9 M

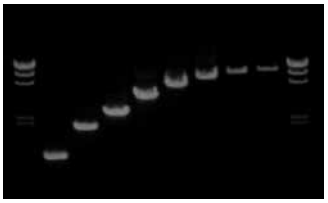


1. 0.5 kb
2. 1 kb
3. 2 kb
4. 4 kb
5. 8 kb
6. 15 kb
7. 20 kb
8. 30 kb
9. 40 kb

M. λ-*Hin*d III digest

cDNA
(Template: equivalent of 250 ng total RNA/50 μl reaction)

M 1 2 3 4 5 6 7 8 M



1. Dystrophin 1 kb
2. Dystrophin 2 kb
3. CCND2 2.8 kb
4. TFR 4 kb
5. Dystrophin 6 kb
6. Dystrophin 8 kb
7. Dystrophin 12 kb
8. Dystrophin 13.5 kb

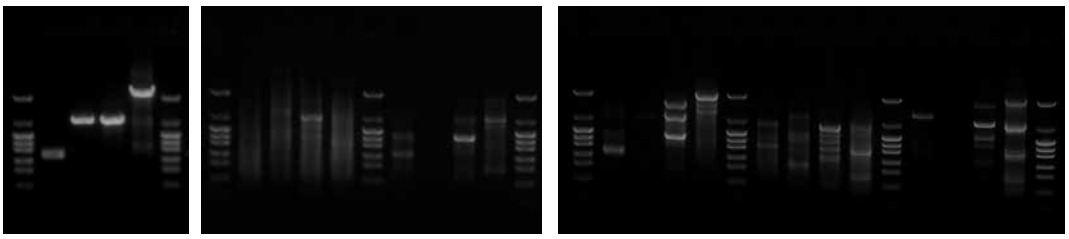
M. λ-*Hin*d III digest

C. Amplification of GC-rich targets

PrimeSTAR GXL DNA Polymerase allows for highly specific amplification of GC-rich templates that are otherwise challenging. Excellent results are achieved without special buffers or reaction conditions. The performance of PrimeSTAR GXL DNA Polymerase in comparison to other commercially available high-fidelity DNA polymerases and polymerases optimized for GC-rich templates is shown below. Reactions were performed according to the protocols specified by each manufacturer.

PrimeSTAR GXL Polymerase | **Company A's enzyme** | **Company B's enzyme** | **Company B's enzyme 2** | **Company C's enzyme** | **Company D's enzyme**

M 1 2 3 4 M M 1 2 3 4 M 1 2 3 4 M M 1 2 3 4 M 1 2 3 4 M M 1 2 3 4 M



Template: Human genomic DNA (100 ng/50 μl reaction)
1. APOE gene 746 bp (GC-content 74%)
2. TGF β1 gene 2,005 bp (GC-content 69%)

Template: *T. thermophilus* HB8 genomic DNA
(10 ng/50 μl reaction)
3. 2,029 bp (GC-content 74%)
4. 4,988 bp (GC-content 74%)

M : pHY Marker

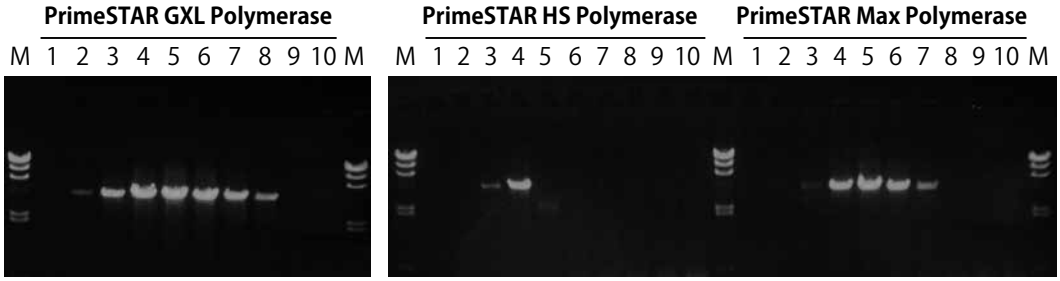
Company B's enzyme 2:
Optimized for GC-rich templates

Company D's enzyme:
Includes buffers optimized for GC-rich templates

D. Sensitivity and range of template quantity

Conventional high-fidelity PCR enzymes are relatively easily affected by excess nucleic acid in the reaction mixture, and frequently do not readily amplify cDNA templates. In contrast, PrimeSTAR GXL DNA Polymerase shows excellent activity over a wide range of template quantities, and therefore, is well-suited to amplification of cDNA templates.

(1) Using cDNA templates obtained by reverse transcription of various quantities of total RNA prepared from HL-60 cells, the transferrin receptor (TFR) gene (4 kb) was amplified using each enzyme in the PrimeSTAR series. Sensitivity and the range of template quantity were compared.



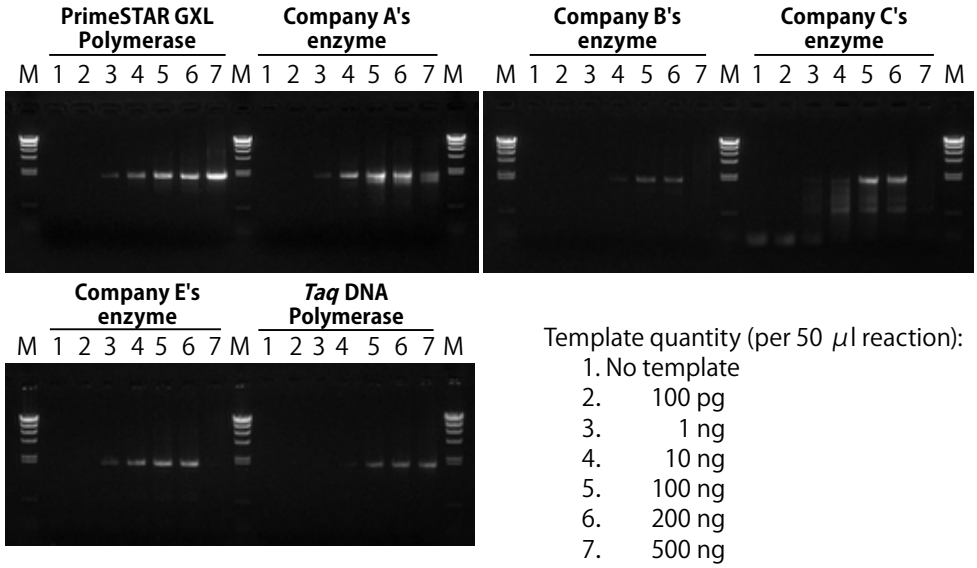
cDNA template quantity (equivalent to total RNA amounts/50 μl reaction):

1. 25 pg
2. 250 pg
3. 2.5 ng
4. 25 ng
5. 250 ng
6. 500 ng
7. 750 ng
8. 1 μg
9. 1.5 μg
10. 2 μg

M. λ-*Hin*d III digest

PrimeSTAR GXL DNA Polymerase demonstrated good amplification over a wide range of template cDNA quantity, as well as excellent sensitivity.

(2) Using various quantities of human genomic DNA as a template, the amplification efficiency of PrimeSTAR GXL DNA Polymerase was compared to the efficiencies of other commercially available high-fidelity PCR enzymes and *Taq* DNA Polymerase. Reactions were performed according to the protocols specified by each manufacturer.



Template: Human genomic DNA
Target: DCLRE 1A gene (2 kb)

Template quantity (per 50 μl reaction):
1. No template
2. 100 pg
3. 1 ng
4. 10 ng
5. 100 ng
6. 200 ng
7. 500 ng
M. λ-*Hin*d III digest

PrimeSTAR GXL DNA Polymerase demonstrated superior sensitivity and amplification efficiency in comparison to other commercially available high-fidelity PCR enzymes and *Taq* DNA Polymerase. High activity was observed for PrimeSTAR GXL DNA Polymerase even in the presence of excess template DNA that suppressed the activity of high-fidelity PCR enzymes from other companies.

IX. Related Products

PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)
PrimeSTAR® HS DNA Polymerase (Cat. #R010A/B)
PrimeSTAR® HS (Premix) (Cat. #R040A)
TaKaRa PCR Thermal Cycler Dice™ Gradient/Standard (Cat. #TP600/TP650)＊
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
NucleoSpin Gel and PCR Clean-Up (Cat. #740609.50/.250)*
Agarose L03 (Cat. #5003)
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)

＊: Not available in all geographic locations. Check for availability in your region.

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