Cat. # R050Q

For Research Use

TaKaRa

PrimeSTAR® GXL DNA Polymerase

Product Manual

v1108Da

Table of Contents

I. Description

PrimeSTAR GXL DNA Polymerase is a revolutionary PCR enzyme that augments the high-fidelity PrimeSTAR HS DNA Polymerase by modification 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 need for extensive optimization of buffers or reaction conditions. In addition, PrimeSTAR GXL DNA Polymerase is compatible with a wide template quantity range, and is capable of robust amplification even in the presence of large excesses of non-target DNA. Excess non-target nucleic acid typically inhibits 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 and 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 range of targets using an extension time of 10 sec./kb.

II. Components (40 reactions) ＊1

PrimeSTAR GXL DNA Polymerase (1.25 U/µl)	40 µl
5X PrimeSTAR GXL Buffer (Mg^{2+} plus) ＊2	400 µl
dNTP Mixture（2.5 mM each）	180 µl

＊1 : Assuming a 50 µl reaction volume
＊2 : 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 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 V.3. Optimization of Parameters	
PrimeSTAR GXL DNA Polymerase	1 µl	1.25 U/50 µl
Sterilized distilled water	to final reaction volume of 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.
68℃＊2 1 min./kb
] 30 cycles [3-step PCR]

- or -

98℃ 10 sec.
68℃＊2 1 min./kb
] 30 cycles [2-step PCR]

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

＊: T_m value calculation method:
T_m (℃) = 2(NA + NT) + 4(NC + NG) - 5
where N represents the number of primer nucleotides 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 smeared or non-specific band(s) are observed during electrophoresis analysis, refer to 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 V.3. Optimization of Parameters	
PrimeSTAR GXL DNA Polymerase	2 µl	2.5 U/50 µl
Sterilized distilled water	to final reaction volume of 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	10 sec./kb	⎭

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

＊: T_m value calculation method:

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

where N represents the number of primer nucleotides 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.	⎫
68℃	20 sec./kb	⎭ 30 cycles [2-step PCR]
- or -		
98℃	10 sec.	⎫
60℃	15 sec.	⎬ 30 cycles [3-step PCR]
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 smeared or non-specific band(s) are observed in electrophoresis analysis, refer to 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 T_m value of ≧ 55℃ (as calculated using the formula in IV. PCR Conditions) or greater than 25-mer in length may provide optimal results. See IV. Protocols.

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

[For >10 kb products]

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

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

[For GC-rich amplification products]
Design primers to have T_m 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 un-chelated (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 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, Cloning, and Sequencing 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 : Use of 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. Accordingly, they can be cloned directly into blunt-end vectors. If necessary, phosphorylate amplified products before cloning. Use 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 NucleoSpin® Extract II (Clontech Cat. #740609.10/.50/.250). Particularly for 3'-protruding restriction enzymes such as *Pst* I, the 3'-protruding termini produced by these enzymes may be deleted by 3' → 5' exonuclease activity of PrimeSTAR GXL DNA Polymerase, if residual polymerase remains present in the restriction digest reaction.

(4) Direct sequencing

Perform phenol/chloroform extraction of PCR products prior to direct sequencing to ensure inactivation of 3' → 5' exonuclease activity. Alternatively, NucleoSpin® Extract II (Clontech Cat. #740609.10/.50/.250) may be used to purify DNA prior to sequencing.

VII. Troubleshooting

Event	Possible Causes	Action
No amplification or poor amplification efficiency	Primer T_m	Refer to 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 Rapid PCR Protocol
	Number of cycles	Set to 35 to 40 cycles
	Purity and quantity of template DNA	Use an appropriate amount of template DNA. Purify the template DNA.
Electrophoresis analysis shows smeared band(s) or extra band(s)	Primer T_m	Refer to 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 T_m 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	Set to 25 to 30 cycles
	Template DNA purity	Purify the template DNA

VIII. Features

A. Accuracy

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

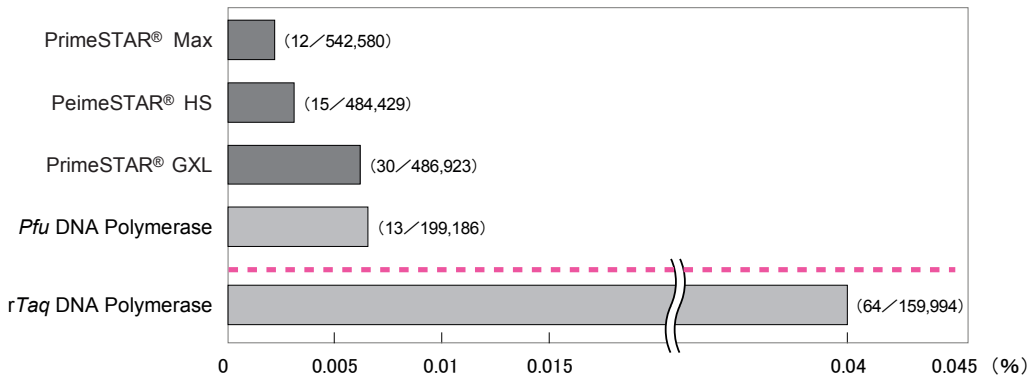
[Method]

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

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

[Result]

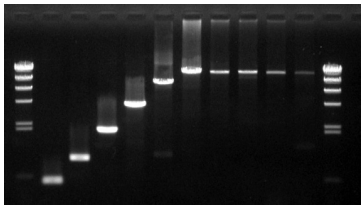
Sequence analysis of DNA fragments amplified using PrimeSTAR GXL DNA Polymerase demonstrated 30 mismatched bases per 486,923 total bases. This is higher fidelity than *Pfu* DNA polymerase.



B. Length of amplification products

PrimeSTAR GXL DNA Polymerase enables 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 template, fragments up to 40 kb in length using λ DNA as template, and fragments up to 13.5 kb in length using cDNA as template.

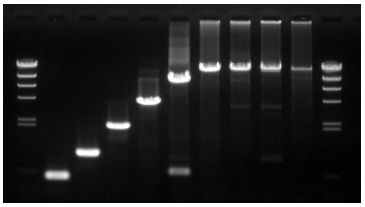
Human genomic DNA
(Template: 100 ng/50 µl reaction)



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

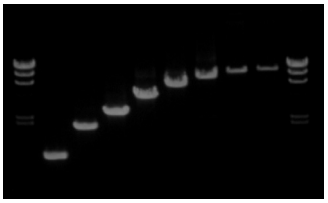
λDNA
(Template: 1 ng/50 µl reaction)



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

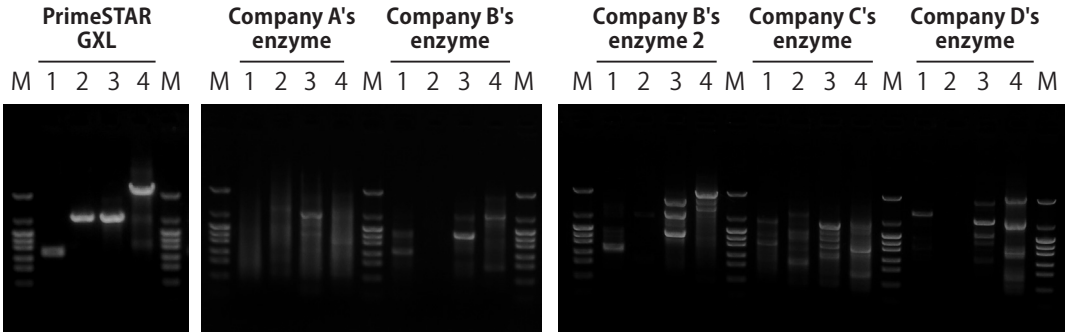


1. Dystrophin 1 kb
2. Dystrophin 2 kb
3. CCND2 2.8 kb
4. TFR 4 kb
5. Dystrophin 6 kb
6. Dystrophin 8 kb
7. Dystrophin 12 kb
8. Dystrophin 13.5 kb

M. λ-*Hin*d III digest

C. Amplification of GC-rich targets

PrimeSTAR GXL DNA Polymerase allows highly specific amplification of GC-rich templates that are otherwise challenging. Excellent results are achieved without requiring 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. Reactions were performed according to the protocols specified by each manufacturer.



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

Template: *T. thermophilus* HB8 genomic DNA
(10 ng / 50 µl reaction)
3. 2029 bp (GC content 74%)
4. 4988 bp (GC content 74%)

M : pHY Marker

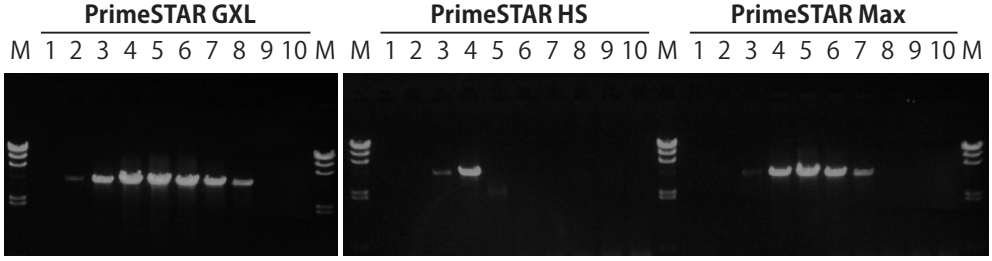
Company B's enzyme 2:
Optimized for GC-rich templates

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

D. Sensitivity and wide template quantity range

Conventional high-fidelity PCR enzymes are relatively easily affected by excess nucleic acid in a reaction solution, 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 for efficient amplification of cDNA templates.

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

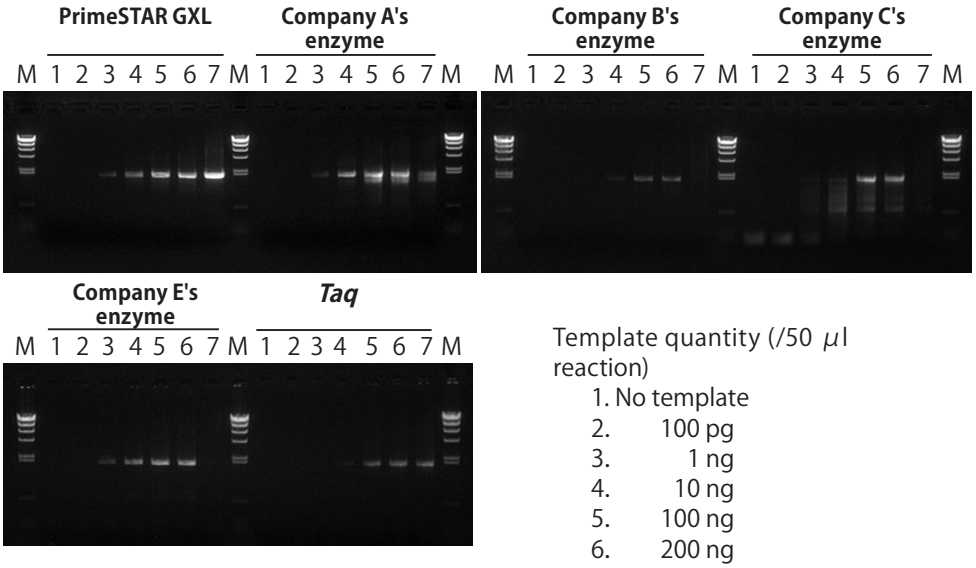


cDNA template quantity (equivalent to total RNA amounts indicated / 50 µl reaction) is as follows:

1.	25 pg	7.	750 ng
2.	250 pg	8.	1 µg
3.	2.5 ng	9.	1.5 µg
4.	25 ng	10.	2 µg
5.	250 ng	M.	λ-*Hin*d III digest
6.	500 ng		

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 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 (/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*. 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® GXL DNA Polymerase (Cat. #R050A)
PrimeSTAR® HS DNA Polymerase (Cat. #R010A/B)
PrimeSTAR® HS (Premix) (Cat. #R040A)
PrimeSTAR® Max DNA Polymerase (Cat. #R045A)
TaKaRa PCR Thermal Cycler Dice™ Gradient/Standard (Cat. #TP600/TP650) ＊
Mighty Cloning Reagent Set (Blunt end) (Cat. #6027)
NucleoSpin® Extract II (Clontech Cat. #740609.10/.50/.250)

＊: not available in the U.S.

NOTICE TO PURCHASER : LIMITED LICENSE

[P1] PCR Notice
Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claims, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Fostcr City, California 94404, USA.

[L15] Hot Start PCR
Licensed under U.S. Patent No. 5.338,671 and 5,587,287, and corresponding patents in other countries.

[M54] PrimeSTAR HS DNA Polymerase
This product is covered by the claims of U.S. Patent No. 7,704,713 and its foreign counterparts.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product web page at http://www.takara-bio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements. If you require licenses for other use, please contact us by phone at +81 77 543 7247.