Cat. # R045A

For Research Use

TaKaRa

PrimeSTAR® Max DNA Polymerase

Product Manual

v1108Da

Table of Contents

I. Description

PrimeSTAR Max DNA Polymerase is a unique high-performance DNA polymerase that possesses the fastest extension speed available, along with the extremely high accuracy, high sensitivity, high specificity, and high fidelity of PrimeSTAR HS DNA Polymerase. High priming efficiency and extension efficiency greatly reduces the time required for annealing and extension steps, faciltating exceptionally fast high-speed PCR reactions. In addition, standardization of extension step time makes PrimeSTAR Max DNA Polymerase suitable for reactions with large amounts of template DNA that would ordinarily be difficult to amplify. Furthermore, an antibody-mediated hot start formulation prevents false initiation events during the reaction assembly due to mispriming and primer digestion. Since PrimeSTAR Max DNA Polymerase is configured as a 2-fold premix containing reaction buffer and dNTP mixture, it allows quick preparation of reactions and is useful for high-throughput applications.

II. Components (for 100 reactions)

PrimeSTAR Max Premix (2X) 625 µl x 4

＊ Mg^{2+} concentration: 2 mM (2X)

III. Storage

–20℃

Note: Repeated freeze-thaw of the enzyme may reduce its activity.

IV. General Composition of PCR Reaction Mixture

		Final conc.
PrimeSTAR Max Premix (2X)	25 µl	1X
Primer 1	10 - 15 pmol	0.2 - 0.3 µM
Primer 2	10 - 15 pmol	0.2 - 0.3 µM
Template	< 200 ng ＊	
Sterilized distilled water	to final reaction volume of 50 µl	

＊： Refer to VI. Optimization of Parameters

Caution: The PCR reaction mixture can be prepared at room temperature. However, keep each of the reaction components on ice during the preparation process.

V. PCR Conditions

When performing rapid amplification protocols using PrimeSTAR Max DNA Polymerase, 3-step reactions are recommended for best results and longest amplification products.

(A) For reactions in which the quantity of template is 200 ng / 50 μl or less:*

98℃	10 sec.	] 30 - 35 cycles
55℃	5 sec. or 15 sec.	
72℃	5 sec./kb	

(B) For reactions in which the quantity of template exceeds 200 ng / 50 μl:*

98℃	10 sec.	] 30 - 35 cycles [3-step PCR]
55℃	5 sec. or 15 sec.	
72℃	30 - 60 sec./kb	

or

98℃	10 sec.	] 30 - 35 cycles [2-step PCR]
68℃	30 - 60 sec./kb	

＊: For rapid amplification protocols (extension step of 5 to 10 sec./kb) with cDNA as template, use a quantity of template that is to equal to or less than the equivalent of 125 ng of total RNA / 50 μl reaction.

If larger quantites of cDNA template are desired, by setting a longer extension time (up to 1 min./kb), it is possible to use up to the equivalent of 750 ng total RNA / 50 μl reaction.

See VII.C. Template Quantity and Reaction Speed Using cDNA as Template.

- Denaturing conditions: 98℃ for 5 to 10 sec. is recommended. If performing denaturation at 94℃, set the denaturation step for 10 to 15 sec.
- Annealing temperature: Use 55℃ as the default annealing temperature.
- Annealing time: For primers that are 25-mer or shorter:

  For primer T_m values (calculated by the formula below) of 55℃ or greater, anneal for 5 sec.

  For primer T_m values (calculated by the formula below) less than 55℃, anneal for 15 sec.

  For primers longer than 25-mers:

  Use an annealing time of 5 sec.

＊ Tm value calculation method:

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

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

Important note:

Because the priming efficiency of PrimeSTAR Max DNA Polymerase is extremely high, use an annealing time of 5 sec. or 15 sec. Longer annealing times may cause smearing of PCR products visible during electrophoresis analysis.

If smearing occurs when performing a 3-step PCR protocol, try a 2-step PCR protocol. See VI. Optimization of Parameters and IX. Troubleshooting.

VI. Optimization of Parameters

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

(1) Template DNA

Recommended quantities of template DNA (50 μl reaction) for rapid amplification protocols (extension step of 5 sec./kb):

Human genomic DNA	5 ng - 200 ng
E. coli genomic DNA	100 pg - 200 ng
λ DNA	10 pg - 10 ng
Plasmid DNA	10 pg - 1 ng

When using more than 200 ng of DNA as template in a 50 μl reaction, use an extension time of 30 to 60 sec./kb for best results.

For rapid amplification protocols (extension time of 5 to 10 sec./kb) with cDNA as template, set the template cDNA quantity to ≦ the equivalent of 25 to 125 ng total RNA / 50 μl reaction.

See VI. C. Template Quantity and Reaction Speed Using cDNA as Template.

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

(2) Amplified Product Sizes

Amplification product sizes using an extension time of 5 sec./kb (for genomic DNA templates) or 5 to 10 sec./kb (for cDNA templates):

Human genomic DNA	up to 6 kb
E. coli genomic DNA	up to 10 kb
cDNA	up to 6 kb
λ DNA	up to 15 kb

When amplifying targets in excess of these lengths, try using an extension time of 15 to 30 sec./kb. In such instances, amplification is affected by the quantity, quality, and sequence composition of the template.

(3) Primer and PCR Conditions

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

For general amplification, 20 to 25-mer primers are suitable. When amplifying longer products, the use of 25 to 30-mer primers may improve results. See section V. PCR Conditions.

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

(4) Annealing conditions

Select annealing conditions as described in V. PCR Conditions. If low product yield occurs, try the following:

<If smearing and/or extra bands appear on agarose electrophoresis gels>

(1) Shorten the annealing time. If performing at 15 sec., set to 5 sec.
(2) If the annealing step has already been set to 5 sec., raise the annealing temperature to 58℃ - 63℃.
(3) Perform 2-step PCR.

<If the target product is not amplified>

(1) Lengthen the annealing time. If performing at 5 sec., set to 15 sec.
(2) Lower the annealing temperature to 50℃ - 53℃.

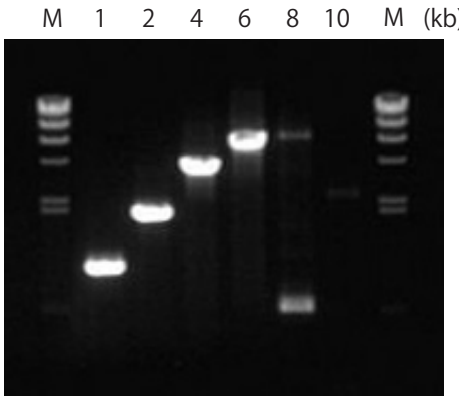
VII. Features

A. Rapid Amplification

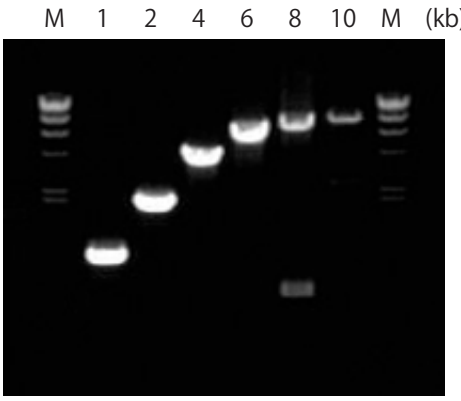
(1) With λ DNA as template, amplification of products ranging in size from 1 to 10 kb was performed using an annealing time of 5 sec. and an extension time of either 10 or 30 sec.

Template	λ DNA [1 ng/50 μl reaction]
Thermal cycler	TaKaRa PCR Thermal Cycler Dice (not available in the U.S.)
PCR conditions	98℃ 10 sec. 55℃ 5 sec. 72℃ 10 or 30 sec.] 30 cycles

[Extension time: 10 sec]



[Extension time: 30 sec]



M: λ-*Hin*d III digest

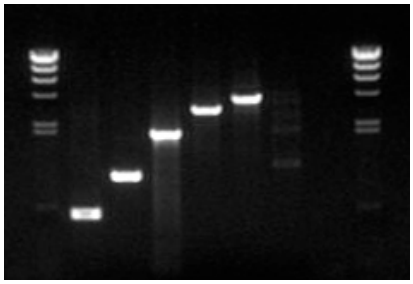
Good amplification is observed for products up to 6 kb in length using an extension time of 10 sec and for products up to 8 kb in length using an extension time of 30 sec. When λ DNA is used as template, extension time of 5 sec./kb may be suitable.

(2) With human genomic DNA as template, amplification of products ranging in size from 0.5 kb to 7.5 kb was performed using an annealing time of 5 sec. and an extension time of either 10 or 30 sec.

Template	Human genomic DNA [100 ng / 50 μl reaction]
Thermal cycler	TaKaRa PCR Thermal Cycler Dice (not available in the U.S.)
PCR conditions	98℃ 10 sec. 55℃ 5 sec. 72℃ 10 or 30 sec.] 30 cycles

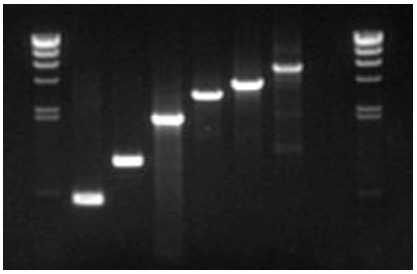
[Extension time: 10 sec]

M 0.5 1 2 3 4 6 7.5 M (kb)



[Extension time: 30 sec]

M 0.5 1 2 3 4 6 7.5 M (kb)



M: λ-*Hin*d III digest

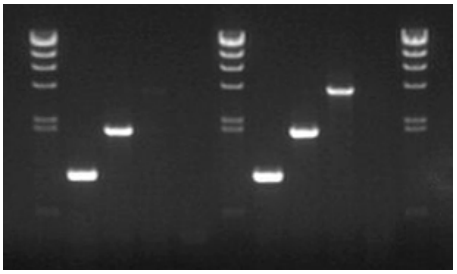
Good amplification is observed for products up to 4 kb in length using an extension time of 10 sec, and for products up to 6 kb in length using an extension time of 30 sec. With human genomic DNA as template, an extension time setting of 5 sec./kb may be suitable.

(3) With cDNA template, amplification of products ranging in size from 1 kb to 6 kb was performed using an annealing time of 15 sec. and an extension time of either 10 or 30 sec.

Template	cDNA [equivalent to 100 ng total RNA) / 50 μl reaction]		
Thermal cycler	TaKaRa PCR Thermal Cycler Dice (not available in the U.S.)		
PCR conditions	98℃	10 sec.	30 cycles
	55℃	15 sec.	
	72℃	10 or 30 sec.	

[Extension time] 10 sec 30 sec

M 1 2 4 6 M 1 2 4 6 M (kb)



M: λ-*Hin*d III digest

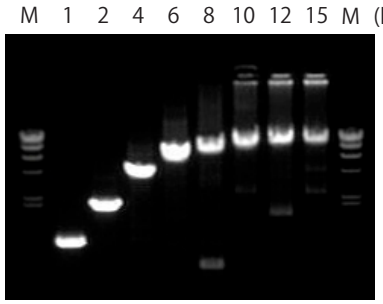
Good amplification was observed for products up to 2 kb in length using an extension time of 10 sec. and for products up to 4 kb using an extension time of 30 sec. With cDNA template, an extension time of 5 to 10 sec./kb is required.

B. Length of amplification products

With λ DNA, *E. coli* genomic DNA, human genomic DNA, or cDNA as the template, amplification sizes of various DNA fragments were examined using an annealing time of 5 sec. or 15 sec and an extension time of 5 sec./kb (genomic DNA) or 10 sec./kb (cDNA).

Template:	λ DNA		1 ng
	E. coli genomic DNA		50 ng
	Human genomic DNA		100 ng
	cDNA		equivalent to 100 ng total RNA
Thermal cycler:	TaKaRa PCR Thermal Cycler Dice		
PCR conditions:	98℃	10 sec.	⎫
	55℃	5 or 15 sec.	⎬ 30 cycles
	72℃	5 (or 10) sec./kb	⎭

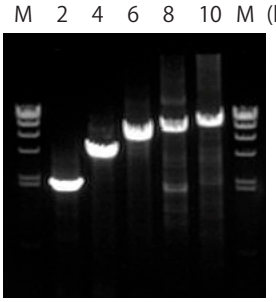
[λ DNA]



M: λ-*Hin*d III digest

Good amplification of products up to 15 kb in length was observed using an extension time of 5 sec./kb.

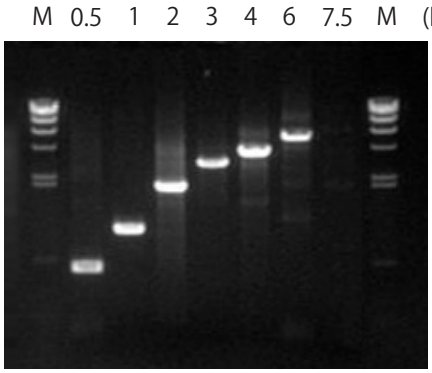
[*E. coli* genomic DNA]



M: λ-*Hin*d III digest

Good amplification of products up to 10 kb in length was observed using an extension time of 5 sec./kb.

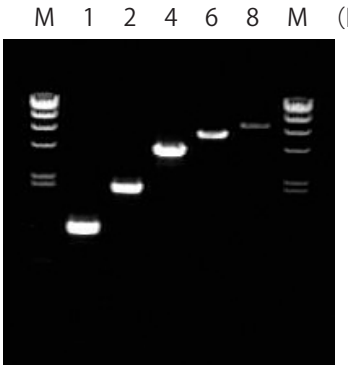
[Human genomic DNA]



M: λ-*Hin*d III digest

Good amplification was of products up to 6 kb in length was observed using an extension time of 5 sec./kb.

[cDNA]

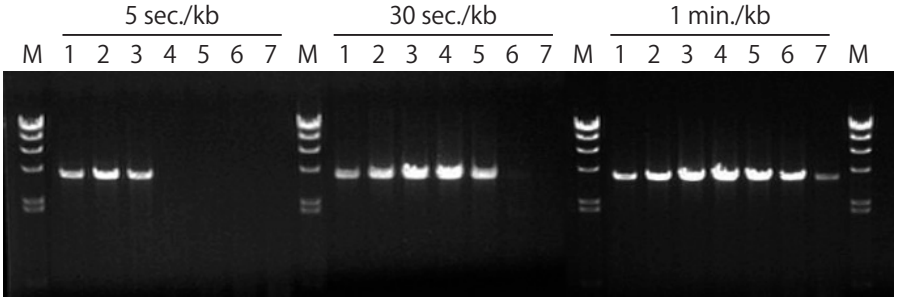


M: λ-*Hin*d III digest

Good amplification of products up to 6 kb in length was observed using an extension time of 10 sec./kb.

C. Template quantity and reaction rate using cDNA as template

Amplification of transferrin receptor (TFR) 4 kb in length was performed with cDNA as template. cDNA was obtained by reverse transcription of various amounts of total RNA, as indicated. The extension times were set to 20 sec (5 sec./kb), 2 min (30 sec./kb) or 4 min (1 min./kb), and the amplification efficiencies were compared.



Template quantity (50 μl reaction)

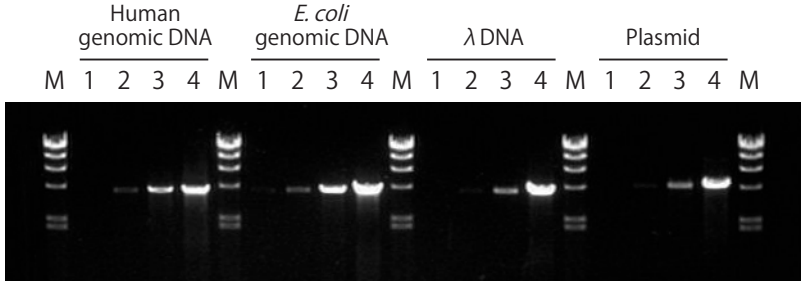
1 : cDNA equivalent to 25 ng total RNA
2 : cDNA equivalent to 50 ng total RNA
3 : cDNA equivalent to 125 ng total RNA
4 : cDNA equivalent to 250 ng total RNA
5 : cDNA equivalent to 500 ng total RNA
6 : cDNA equivalent to 750 ng total RNA
7 : cDNA equivalent to 1 μg total RNA
M: λ-*Hin*d III digest

For rapid amplification protocols using an extension time of 5 sec./kb, it is necessary to use cDNA template that is ≦ the equivalent of 125 ng total RNA / 50 μl reaction. When using longer extension times (up to 1 min./kb), the quantity of cDNA template can be increased up to the equivalent of 750 ng total RNA / 50 μl reaction.

D. Sensitivity

With various amounts of human genomic DNA, *E. coli* genomic DNA, λ DNA, or plasmid DNA as template, sensitivity was examined when amplification of a 4 kb DNA fragment was performed using an extension time of 20 sec.

Thermal cycler	TaKaRa PCR Thermal Cycler Dice		
PCR conditions	98℃	10 sec.	30 cycles
	55℃	5 sec.	
	72℃	20 sec.	



M : λ-*Hin*d III digest

Template quantity:

	Lane 1	Lane 2	Lane 3	Lane 4
Human genomic DNA	100 pg	1 ng	10 ng	100 ng
E. coli genomic DNA	1 pg	10 pg	100 pg	1 ng
λ DNA	100 fg	1 pg	10 pg	100 pg
Plasmid DNA	100 fg	1 pg	10 pg	100 pg

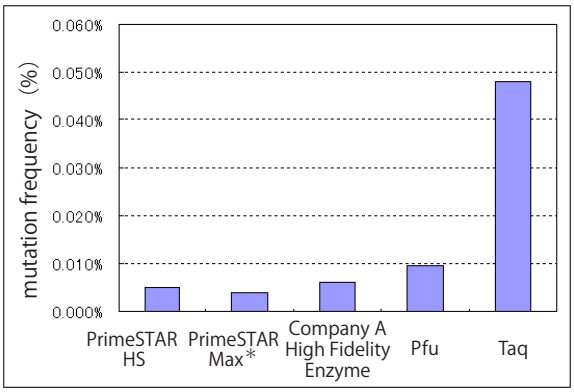
E. Accuracy

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

[Method] Eight arbitrarily selected GC-rich regions were amplified with PrimeSTAR Max DNA Polymerase or other DNA polymerases, using *Thermus thermophilus* HB8 genomic DNA as template.
PCR products (approx. 500 bp each) were each 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 Max DNA Polymerase demonstrated only 9 mismatched bases per 230,129 total bases. This is higher fidelity than an alternative high-fidelity enzyme from Company A, and 10-fold higher fidelity than *Taq* DNA polymerase.

Fidelity comparison of each enzyme



*: Out of 230,129 analyzed bases that were amplified using PrimeSTAR Max DNA Polymerase, only 9 base errors occurred.

VIII. 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 Max 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 Max DNA Polymerase have blunt-end termini. Accordingly, they can be cloned directly into blunt-end vectors. If necessary, phosphorylate the amplified products before cloning. Use of Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) is recommended for cloning into a blunt-end vector.

3) Restriction enzyme reaction
Prior to performing restriction enzyme digestion of amplified PCR products, remove all traces of PrimeSTAR Max 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 Max 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.

IX. Troubleshooting

Event	Possible causes	Action
No amplification or poor amplification efficiency	Extension time	Set to 10 to 60 sec./kb *
	Number of cycles	Set to 35 to 40 cycles.
	Annealing time	Set to 15 sec.
	Annealing temperature	Lower by 2℃ per trial
	Reaction volume	Use 25 μl
	Purity and quantity of template DNA	Use an appropriate amount of template DNA. Purify the template DNA＊.
	Primer concentration	Use 0.2 - 0.5 μM (final conc.)
Electrophoresis analysis shows smeared band(s) or extra band(s)	Annealing time	Set to 5 sec.
	Annealing temperature	Raise by 2℃ per trial up to 63˚C. Try 2-step PCR.
	Template DNA quantity	Use an appropriate amount of template DNA. Do not use more than necessary.
	Number of cycles	Set to 25 to 30 cycles.
	Primer concentration	Use at a final concentartion of 0.2 - 0.3 μM

＊: When using crude samples containing large quantities of RNA, such as samples prepared by thermal lysis, improved results may be achieved by setting the extension time to 60 sec./kb.

X. Related Products

PrimeSTAR® HS DNA Polymerase（Cat. #R010A/B）
PrimeSTAR® HS (Premix) (Cat. #R040A)
PrimeSTAR® GXL DNA Polymerase (Cat. #R050A/B）
PrimeSTAR® Mutagenesis Basal Kit (Cat. #R046A)＊
NucleoSpin® Extract II (Cat. #740609.10/.50/.250）
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
TaKaRa PCR Thermal Cycler Dice™ Gradient/Standard (Cat. #TP600/TP650)＊

＊: not available in the U.S.