Cat. # R044A

For Research Use

TaKaRa

PrimeSTAR® HS DNA Polymerase with GC Buffer

Product Manual

v201908Da

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I. Description

PrimeSTAR HS DNA Polymerase is a unique high fidelity DNA polymerase that additionally offers high amplification efficiency for PCR amplification. PrimeSTAR HS possesses a robust 3′ → 5′ exonuclease activity, resulting in superior proofreading activity and a low error rate. It also has high amplification efficiency (superior to that of *Taq* DNA polymerase). Furthermore, presence of a monoclonal antibody in the reaction mixture suppresses both the DNA polymerase and 3′ → 5′ exonuclease activities prior to the first denaturing step, preventing false initiation events during reaction assembly due to mispriming and primer digestion. Finally, PrimeSTAR's high priming efficiency makes it possible to shorten reaction times by reducing the length of the annealing step.

PrimeSTAR HS DNA Polymerase with GC Buffer was developed for accurate amplification of GC rich targets. With PrimeSTAR GC Buffer, amplifications exhibit both the high fidelity and high amplification efficiency expected from PrimeSTAR HS DNA Polymerase, and additionally yield excellent results in high-specificity applications such as amplification of GC rich DNA templates.

II. Components (for 200 reactions)

PrimeSTAR HS DNA Polymerase (2.5 U/μl)	100 μl
2X PrimeSTAR GC Buffer (Mg^{2+} plus) ＊	1.7 ml x 3
dNTP Mixture (2.5 mM each)	800 μl

＊ Mg^{2+} concentration is 2 mM (2X)

III. Storage - 20℃

IV. General PCR Reaction Mixture (50 μl volume)

Reagent	Volume/Amount	Final Conc.
2X PrimeSTAR GC Buffer (Mg^{2+} plus)	25 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 DNA	< 200 ng	
PrimeSTAR HS DNA Polymerase (2.5 U/μl)	0.5 μl	1.25 U/50 μl
Sterile purified water	up to 50 μl	

＊ The PCR reaction mixture can be prepared at room temperature. However, the enzyme and other reagents should be kept on ice during operation.

V. PCR Conditions

This kit is designed to perform amplification of GC rich target DNA.
Typically, best results are obtained using a 2-step PCR protocol. However, if this protocol does not yield sufficient product in quality and quantity, the 3-step PCR protocol is recommended. Also, refer to the following sections : VI. Optimization of Parameters and X. Troubleshooting.

(A) 2-Step PCR Protocol

98℃	10 sec	] 30 cycles
68℃	1 min/kb	

(B) 3-Step PCR Protocol

98℃	10 sec	] 30 cycles
60℃	5 sec	
72℃	1 min/kb	

Note: The PrimeSTAR HS has extremely high priming efficiency.
Therefore, when using the 3-step PCR Protocol, the annealing time should be set at only 5 sec. Longer annealing times can result in increased background.

VI. Optimization of Parameters

PrimeSTAR HS DNA Polymerase with GC Buffer is designed to perform amplification of GC rich targets, while exhibiting both the high accuracy and efficiency characteristic of PrimeSTAR HS DNA Polymerase. Optimization of parameters in PCR condition may be required to generate maximum performance.

(1) For best results, use of 1.25 U of enzyme per 50 μl reaction mixture is recommended. However, depending upon the size of the amplified fragment and the purity and amount of template used, the amount of enzyme may need to be modified. For example, if smearing or non-specific banding is observed, results may be improved by reducing the amount of enzyme to ~ 0.625 U/50 μl.

(2) Template DNA
Recommended template DNA amounts (assuming a 50 μl reaction)

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

Note : Avoid using excess amounts of template DNA. The efficiency of reaction can be decrease, particularly when more than 200 ng of template is used. DNA, which contain uracil, cannot be used as a template.

(3) dNTP and Mg^{2+} concentration:
Because dNTPs have a chelative effect, higher dNTP concentrations lower the effective Mg^{2+} concentration of the reaction mixture. The supplied 2X PrimeSTAR GC Buffer provides final concentration of 1 mM Mg^{2+} in the reaction mix, that has been optimized for use with a 200 μM each dNTP concentration. Avoid modifying the dNTP concentration in the reaction mix.
Furthermore, substitution of dTTP with dUTP in a PrimeSTAR HS DNA Polymerase reaction mix is also not recommended as amplification efficiency will be lowered significantly.

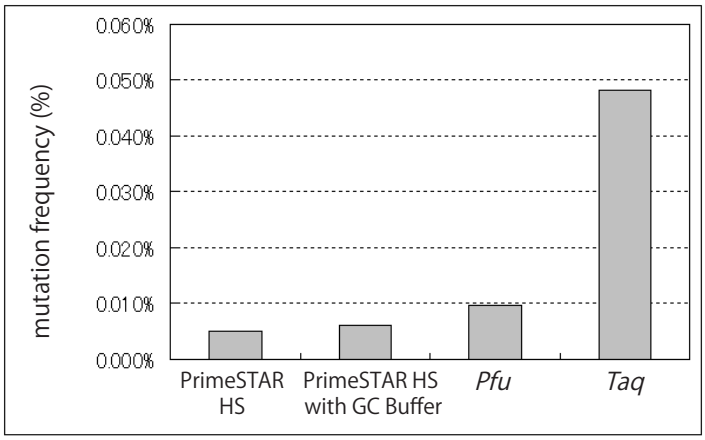
(4) Primer and PCR conditions
Commercially available primer design software, such as OLIGO Primer Analysis Software (Molecular Biology Insights), is recommended for determining appropriate primer sequences. For general amplification reactions, 20 - 25 mer primers should yield satisfactory results.

Avoid the use of primers that contain inosine with PrimeSTAR HS DNA Polymerase. However, it is possible to use degenerate primers with this enzyme.

VII. Fidelity

After PCR amplification of eight randomly selected regions (each about 500 bp in length) of GC rich *Thermus thermophilus* HB8 genomic DNA, PCR products were cloned into a vector. Multiple clones were selected for each product and sequenced, and the mutation frequency was determined.
In this assay, the fidelity of PrimeSTAR HS DNA Polymerase with GC Buffer was higher than that of *Pfu* DNA Polymerase, and was similar to those with PrimeSTAR HS DNA Polymerase (PrimeSTAR Buffer used).
Sequence analysis is the most accurate method for obtaining enzyme fidelity comparisons relevant to the most commonly-used applications. These results demonstrate that PrimeSTAR HS with GC Buffer is a reliable polymerase especially for high fidelity reaction.

Comparison of Enzyme Fidelity



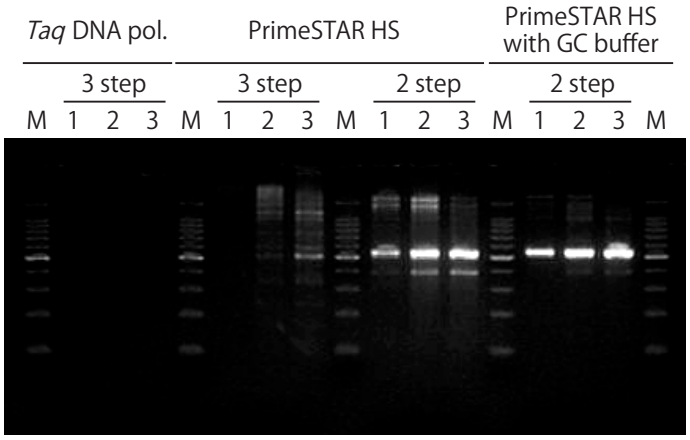
＊ Amplification with PrimeSTAR HS DNA Polymerase with GC Buffer results in only 25 errors out of a total 304,110 bases.

VIII. Amplification Examples

Result 1 :

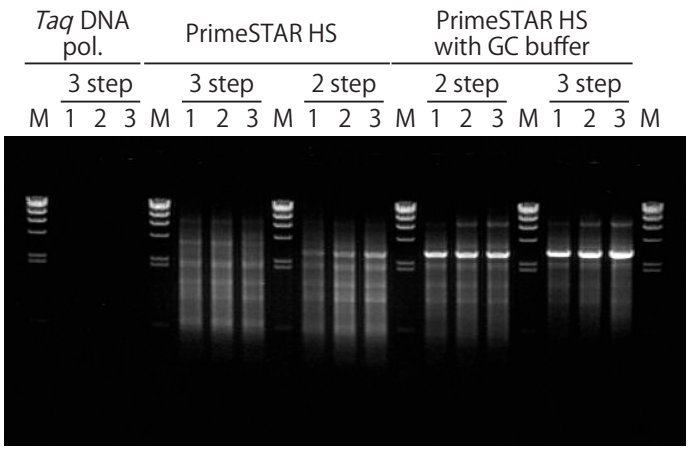
The reactivity of *Taq* DNA Polymerase, PrimeSTAR HS DNA Polymerase, and PrimeSTAR HS DNA Polymerase with GC Buffer were compared for amplification of Human *APOE* gene (520 bp; 74.8% GC) and a region of *Tth*HB8 (3005 bp; 73.2% GC) as targets. The reaction mixture and PCR conditions were according to the recommended protocol of each product.

[Human *APOE* gene 520 bp]



Template:
1 : Human genomic DNA 1 ng
2 : Human genomic DNA 10 ng
3 : Human genomic DNA 100 ng
M : 100 bp DNA Ladder

[*Tth*HB8 3005 bp]



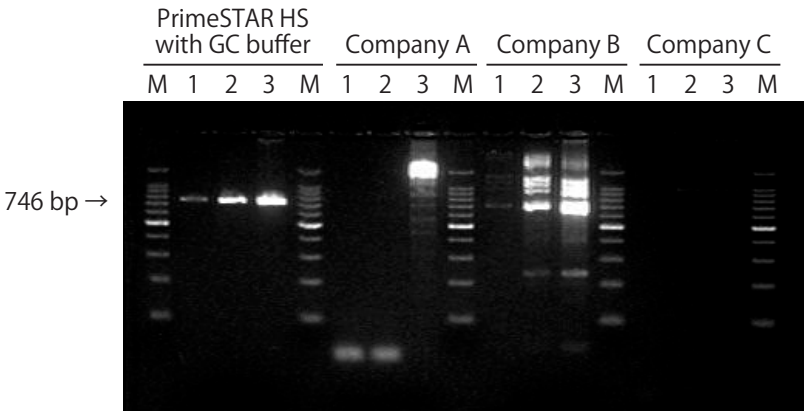
Template:
1 : *Tth* HB8 genomic DNA 100 pg
2 : *Tth* HB8 genomic DNA 1 ng
3 : *Tth* HB8 genomic DNA 10 ng
M : λ -*Hin*d III digest

PrimeSTAR HS DNA Polymerase with GC Buffer provides excellent amplification of GC rich targets in comparison to the other enzymes.

Result 2 :

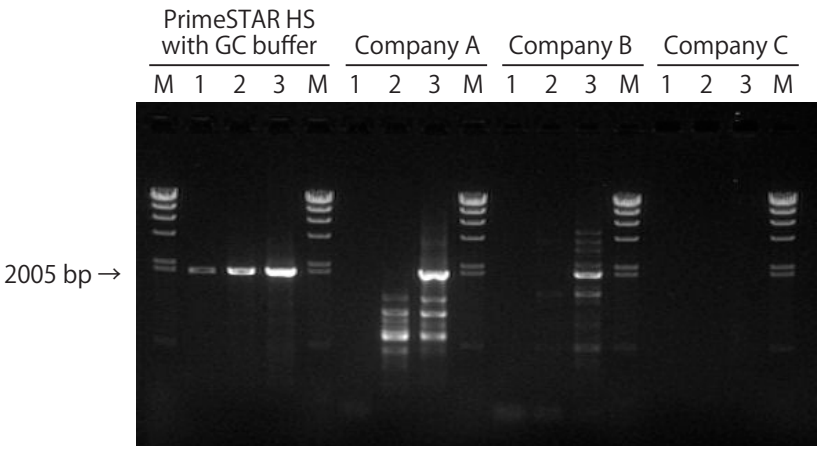
The reactivity of the GC rich high fidelity enzymes of Company A, Company B, and Company C were compared with PrimeSTAR HS DNA Polymerase with GC Buffer for amplification of Human *APOE* (746 bp; 73.9% GC), TGF β 1 (2005 bp; 68.8% GC), and regions of *Tth*HB8 (3005 bp, 73.2% GC, and 5030 bp, 71.2% GC) as targets. Each enzyme was reacted at the recommended reaction mixture and PCR conditions.

[Human *APOE* gene 746 bp]



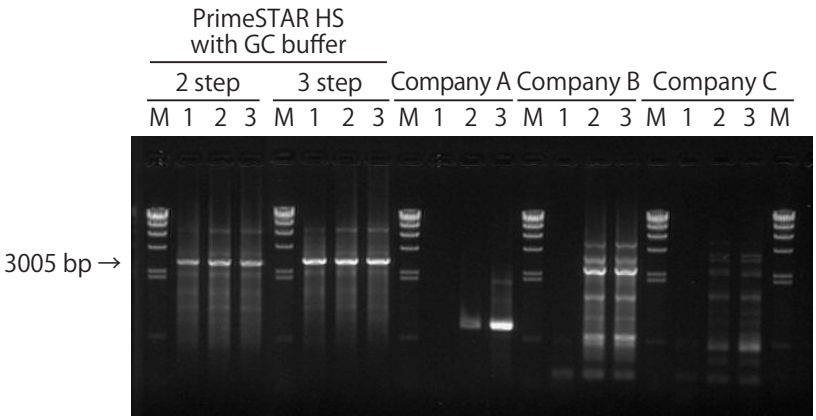
Template:
1 : Human genomic DNA 1 ng
2 : Human genomic DNA 10 ng
3 : Human genomic DNA 100 ng
M : 100 bp DNA Ladder

[Human TGF β1 gene 2005 bp]

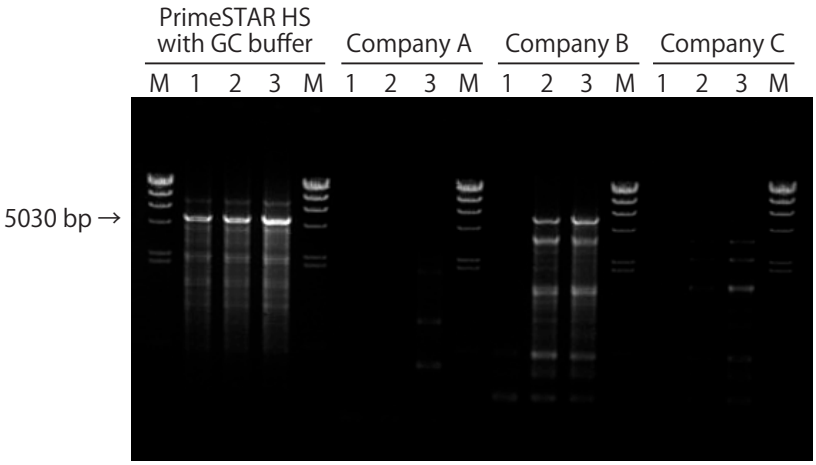


Template:
1 : Human genomic DNA 1 ng
2 : Human genomic DNA 10 ng
3 : Human genomic DNA 100 ng
M : λ -*Hin*d III digest

[*Tth*HB8 3005 bp and 5030 bp]



Template:
1：*Tth* HB8 genomic DNA 100 pg
2：*Tth* HB8 genomic DNA 1 ng
3：*Tth* HB8 genomic DNA 10 ng
M： λ -*Hin*d III digest



Template:
1：*Tth* HB8 genomic DNA 100 pg
2：*Tth* HB8 genomic DNA 1 ng
3：*Tth* HB8 genomic DNA 10 ng
M： λ -*Hin*d III digest

The results show that PrimeSTAR HS DNA Polymerase with GC Buffer provides excellent amplification efficiency with higher specificity than other supplier's GC-rich high fidelity enzymes.

IX. Electrophoresis, Cloning, and Sequencing of Amplified Products

(1) Electrophoresis of amplified products

TAE Buffer is recommended for agarose gel electrophoresis of amplified products that are obtained using PrimeSTAR HS DNA Polymerase with GC Buffer. Use of TBE Buffer may result in DNA banding patterns which become broad at the gel bottom.

(2) Cloning

Most products amplified with PrimeSTAR HS DNA Polymerase with GC Buffer have blunt-end termini. They (if necessary, phosphorylate before cloning) can be cloned directly into blunt-ended vectors. The Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) is recommended for cloning into blunt-ended vectors.

(3) Restriction Enzyme Digestion

Prior to performing restriction enzyme digestion of the PCR products with PrimeSTAR HS DNA Polymerase
with GC Buffer all traces of the polymerase should be removed by phenol/chloroform extraction. In particular, the removal of the polymerase is important to digest with enzymes arising 3′ -protruding cleavage sites, such as *Pst* I as residual PrimeSTAR HS DNA Polymerase 3′ → 5′ exonuclease result in deletion of 3′-protruding region.

(4) Direct sequencing

Phenol/chloroform extraction of PCR products prior to direct sequencing is recommended to ensure inactivation of PrimeSTAR's 3′ → 5′ exonuclease activity.

X. Troubleshooting

Problem : No or poor amplification.

(1) Purity and amount of template DNA
⇒ Use proper amount of template DNA.
⇒ Increase purity of DNA.

(2) Annealing/extension temperature
⇒ Lower temperature in decrements of 2℃.
⇒ Perform using 3-step PCR method.

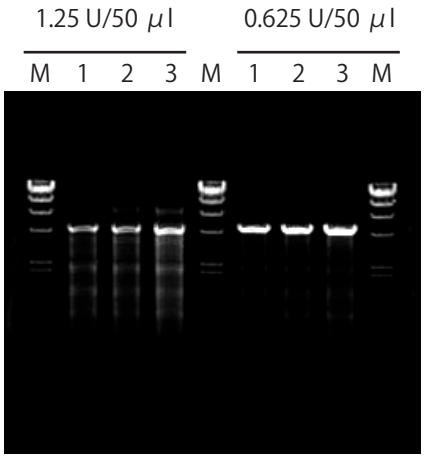
(3) Concentration of Primer
⇒ Test the final primer concentration in a range of 0.2 - 0.5 μM.

(4) Annealing time
⇒ Set the annealing time for 3-step PCR for 15 sec.

Problem: Extra or smearing bands.

(1) Enzyme amount
⇒ Decrease enzyme concentration to ～ 0.625 U/50 μl reaction.

[*Tth* HB8 5030 bp]



(2) Amount of template DNA
⇒ Use an appropriate amount of template DNA. Avoid excessive amounts of template DNA.

(3) Annealing/extension temperature
⇒ Raise the temperature in increments of 2℃

(4) Primer Concentration
⇒ Test the final concentration in the range of 0.2 - 0.3 μM.

(5) Cycle Number
⇒ Set at 25 - 30 cycles

XI. Related Products

PrimeSTAR® HS DNA Polymerase (Cat. #R010A/B)
PrimeSTAR® HS Premix (Cat. #R040A)
PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)
PrimeSTAR® GXL DNA Polymerase (Cat. #R050A/B)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)