



PrimeSTAR[®] HS DNA Polymerase

 [cat info](#)

 [manual](#)

New TaKaRa high fidelity PCR enzyme with high amplification efficiency

Also available with GC buffer and 2X Premix version

High fidelity DNA amplification is a necessity when PCR products need to be cloned, sequenced and expressed. Reliable copy of target DNA can actually save time in many downstream applications to PCR process like mutation study, mutagenesis, library screening, protein expression ... To meet such needs, Takara Bio developed a proprietary Hot Start DNA Polymerase, PrimeSTAR[®], with the highest level of fidelity. PrimeSTAR[®] HS DNA Polymerase has very strong 3'→5' exonuclease activity, providing excellent proofreading activity during DNA amplification, as well as high amplification efficiency exceeding that of *Taq* DNA polymerase. This document shows the excellent performance of PrimeSTAR[®] HS DNA Polymerase and how to determine optimal reaction conditions. Furthermore it provides actual examples of the reaction with this enzyme.

Features

- Highest level of accuracy for PCR.
- Highly efficient amplification exceeds that of *Taq* DNA polymerase.
- GC-rich sequences are amplified efficiently and accurately.
- Superior priming efficiency allows very specific amplification with short annealing time.
- PrimeSTAR[®] consists of an antibody mediated hot start PCR enzyme.

Contents (200 reactions)

PrimeSTAR[®] HS DNA Polymerase (2.5 U/μl) 100 μl
 5 x PrimeSTAR[®] Buffer (Mg²⁺ plus)* (5x) 1 ml x 2
 dNTP Mixture (2.5 mM each) 800 μl

*: Mg²⁺ concentration is 5 mM (5x).

Comparison of accuracy between PrimeSTAR[®] HS DNA Polymerase and various PCR enzymes

Eight arbitrarily selected regions (amplification size: approximately 500 bp each) were PCR-amplified with PrimeSTAR[®] HS DNA Polymerase and 3 PCR enzymes, using GC-rich *Thermus thermophilus* HB8 genomic DNA as a template. Amplification products were cloned into suitable vectors. Multiple clones of each region were selected and sequenced. The error rate was then determined at the sequence level (Fig. 1). PrimeSTAR[®] HS DNA Polymerase showed only 12 incorrect bases in approximately 250,000 total bases, indicating 10-fold higher fidelity than *rTaq*, and great accuracy, exceeding *Thermococcus kodakaraensis*-derived and *Pyrococcus* sp.-derived DNA polymerases (i.e. KOD and *Pfu* respectively), which are known as high fidelity enzymes.

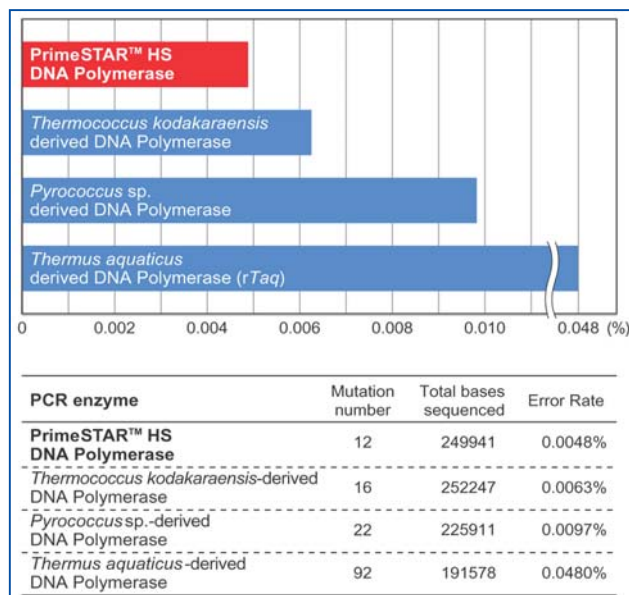


Fig. 1 Comparison of error rate in PCR amplification

Reaction mixtures were prepared and PCR conditions were set according to the recommended protocol of each enzyme (50-μl reaction, 30 cycles).

The 10-fold higher fidelity, compared to *Taq* DNA polymerase, may not appear as a large difference compared to conventional notations calculated by the Kunkel or Cline method, which counts mutant phenotypes; however, these values were obtained in actual sequence analyses. This method for calculation of fidelity is best adapted to actual PCR experiments, where product is sequenced for confirmation. PrimeSTAR[®] HS DNA Polymerase can be used for important reactions requiring accuracy.

Comparison of specificity by annealing time

PrimeSTAR[®] HS DNA Polymerase is mixed with monoclonal antibody to inhibit DNA polymerase activity and 3'→5' exonuclease activity at normal temperature. This suppresses mispriming and primer digestion prior to PCR reaction thus improving specificity of PCR reactions and allowing preparation at room temperature.

Since this enzyme has very high priming efficiency, highly specific amplification is achieved and reaction time can be reduced at the same time by setting a short annealing time (5 or 15 sec). As an example, 0.5 kbp and 4 kbp fragments of human p53 gene were PCR-amplified at different annealing times (Fig. 2).

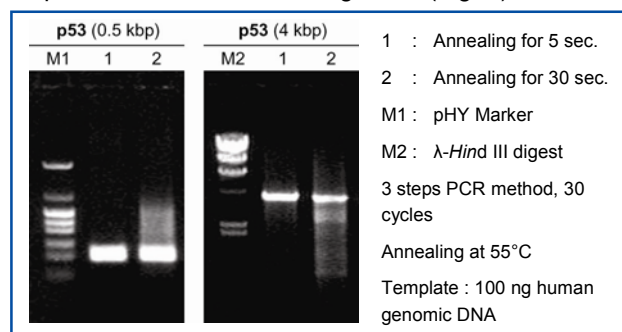


Fig. 2 Comparison of specificity with different annealing time

Comparison of amplification efficiency between PrimeSTAR[®] and competitors' PCR enzymes

PrimeSTAR[®] HS DNA Polymerase not only provides excellent performance of the enzyme itself, but also allows highly sensitive PCR amplification for a broad range of targets with fully optimized reaction buffer.

(1) PCR amplification using a 2 kbp fragment of human DCLRE1A gene as a target

Using a 2 kbp fragment of human DCLRE1A gene as a target, PrimeSTAR[®] HS DNA Polymerase was compared with *rTaq* and competitors' high fidelity enzymes for reactivity (Fig. 3).

PrimeSTAR[®] HS DNA Polymerase showed excellent reactivity with greater specificity and one order higher detection sensitivity than *rTaq* and competitors' high fidelity enzymes.

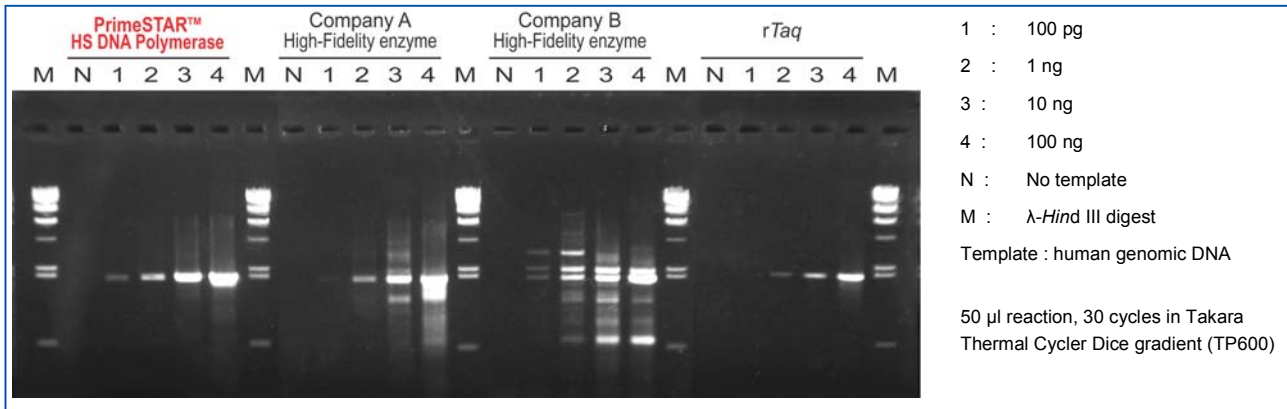


Fig. 3 Comparison of amplification efficiency between PrimeSTAR[®] HS DNA Polymerase and various PCR enzymes
Reaction mixtures were prepared and PCR conditions were set in accordance with the recommended protocol of each enzyme.

(2) PCR amplification using a high GC content region as a target

Using *Thermus thermophilus* HB8 genomic DNA as a template and a GC-rich region (amplification size: 537 bp; GC content: approximately 70%) as a target, PrimeSTAR[®] HS DNA Polymerase was compared with *rTaq* and competitors' high fidelity enzymes for reactivity (Fig. 4).

PrimeSTAR[®] HS DNA Polymerase showed excellent reactivity even for a GC-rich region, with higher detection sensitivity than *rTaq* and competitors' high fidelity enzymes.

In this amplification, PrimeSTAR[®] HS DNA Polymerase had an error rate of 0.0056%, indicating great accuracy. It is particularly adapted to GC rich template amplification with high accuracy.

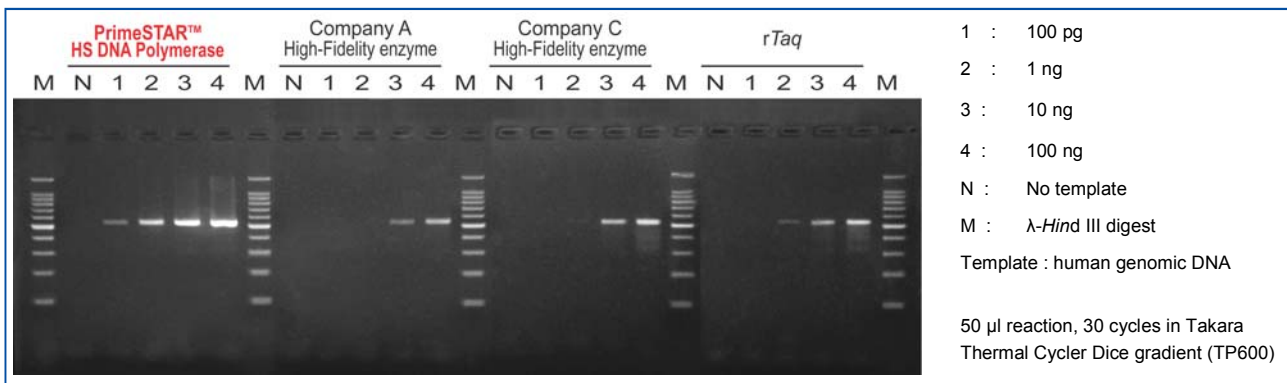


Fig. 4 Comparison of reactivity in a GC rich region
Reaction mixtures were prepared and PCR conditions were set in accordance with the recommended protocol of each enzyme.

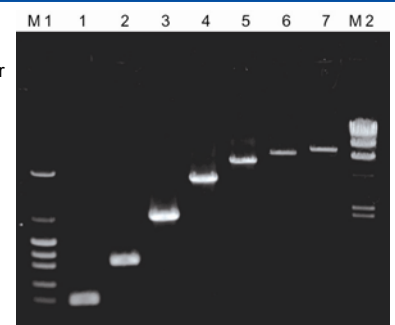
(3) PCR amplification of long-chain DNA
PrimeSTAR[®] HS DNA Polymerase not only has high fidelity and amplification efficiency, but also is suitable for amplification of long-chain DNA. In this example, various sizes of DNA were amplified using human genome (Fig. 5) and *E. coli* genome (Fig. 6) as templates.

Fig. 5 Amplification of human genome DNA using the PrimeSTAR[®] HS DNA Polymerase
(50ng template DNA / 50µl reaction, 30 cycles in Takara Thermal Cycler Dice Gradient TP600)

1: 0.5 kb	4: 4 kb	7: 8.5 kb
2: 1 kb	5: 6 kb	M1: pHY Marker
3: 2 kb	6: 7.5 kb	M2: λ -Hind III

PCR cycle conditions (30 cycles):

size : 0.5-6 kb	size : 7.5-8.5 kb
98°C 10 sec.	98°C 10 sec.
60°C 5 sec.	68°C 8 min.
72°C 1 min.	



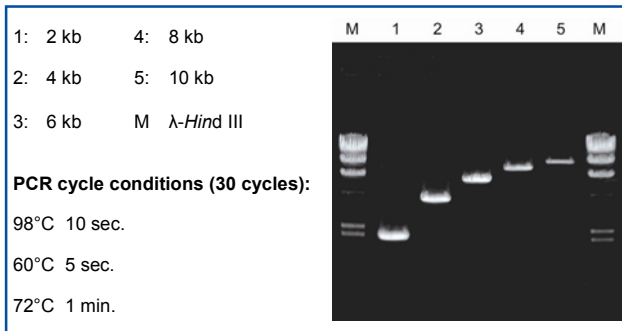


Fig. 6 Amplification of *E. coli* genome DNA using the PrimeSTAR[®] HS DNA Polymerase (100pg template DNA / 50µl reaction, in Takara Thermal Cycler Dice Gradient TP600)

Excellent amplification was observed for at least 8.5 kp of human genome and at least 10 kb of *E. coli* genome. This suggests that long chains can be amplified efficiently even using genomes of important complexity and high secondary structure.

How to determine reaction conditions

◆ General PCR reaction mixture composition (total 50 µl)

	Amount used	Final concentration
5XPrimeSTAR [®] Buffer (Mg ²⁺ plus)	10 µl	1X
dNTP Mixture (2.5 mM each)	4 µl	200 µM each
Primer 1	10 to 15 pmol	0.2 to 0.3 µM
Primer 2	10 to 15 pmol	0.2 to 0.3 µM
Template	< 200 ng*	
PrimeSTAR [®] HS (2.5 U/µl)	0.5 µl	1.25 U/50 µl
Sterile distilled water		up to 50 µl

PCR reaction mixtures can be prepared at room temperature. Enzymes and other reagents should be kept on ice.

*Optimal amounts of template DNA are shown below (for 50-µl reaction):

Human genomic DNA	5 to 200 ng (< 200 ng)
<i>E. coli</i> genomic DNA	100 pg to 100 ng
IDNA	10 pg to 10 ng
Plasmid DNA	10 pg to 1 ng

Do not use an excess amount of template DNA, as this might decrease reactivity.

◆ PCR conditions

● For 3-step PCR

98°C	10 sec	30 cycles
55°C	5 or 15 sec	
72°C	1 min/kb	

● For 2-step PCR (shuttle)

98°C	10 sec	30 cycles
68°C	1 min/kb	

For amplification using PrimeSTAR[®] HS DNA Polymerase, 3-step PCR is basically recommended.

● Denaturation conditions

Denaturation at 98°C for 5 to 10 sec is recommended. For denaturation at 94°C, set to 10 to 15 sec.

● Annealing temperature

Try at 55°C first.

● Annealing time

When $T_m^* \geq 55^\circ\text{C}$: → Set to 5 sec.

When $T_m^* < 55^\circ\text{C}$: → Set to 15 sec.

*: $T_m (\text{°C}) = 2 (\text{NA} + \text{NT}) + 4 (\text{NC} + \text{NG}) - 5$

Apply the above rule when the primer is not longer than 25 mer. If the primer is longer than 25 mer, set the annealing time to 5 sec.

If the 3-step reaction is smeared or primers T_m value is not less than 70°C, try the 2-step PCR.

Amplification under the same PCR conditions

Using human genome as a template, various lengths of target gene were amplified under the same PCR conditions.

Template: Human genomic DNA 100 ng/50 µl reaction
 PCR conditions: 98°C, 10 sec
 68°C, 8 min 30 cycles

(in TaKaRa Thermal Cycler Dice Gradient, cat. # TP600)

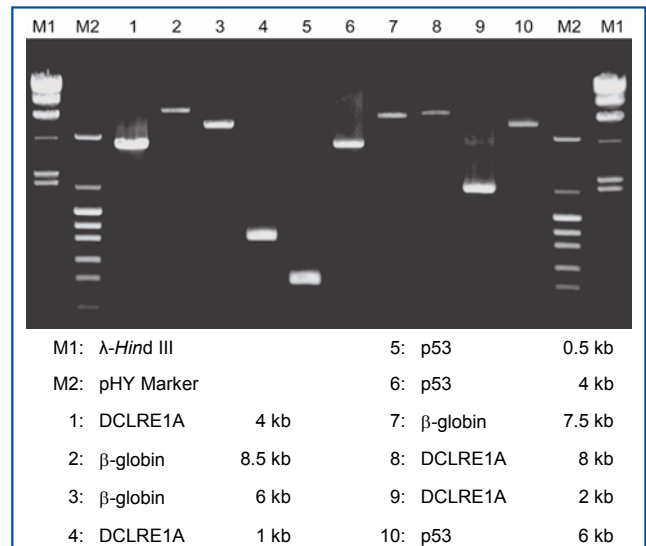


Fig. 7 Amplification of various sizes under the same PCR conditions

Various targets from 0.5 to 8.5 kbp could be amplified under the same PCR conditions. This enzyme, being highly accurate, is furthermore suitable for cloning from libraries and other demanding PCR applications.

As shown above, PrimeSTAR[®] HS is a very convenient enzyme with higher amplification efficiency and fidelity than conventional high fidelity enzymes. We are convinced that this enzyme helps your research.

Related products

Most of the PCR products amplified using PrimeSTAR[®] HS DNA Polymerase are blunt-ended. Therefore, PCR products can be cloned directly, or through phosphorylation if necessary, to blunt-ended vectors but not into T-vectors.

For cloning to blunt-ended vectors, the Mighty Cloning Kit (Blunt End) sold separately, is recommended.




The Mighty Cloning Kit allows blunting and phosphorylation of any PCR products in one step, without prior purification. After reaction, the solution is treated in a Micropure®-EZ (Millipore) centrifuge tube with a protein removal filter, and the filtrate is used for ligation. The use of Ligation Mighty Mix, achieves easy and efficient cloning into dephosphorylated blunt-ended vectors (pUC118) provided.

Components of Mighty Cloning Kit (Blunt End)

- Reagent Set for Mighty Cloning Kit (Blunt End) 20 reactions
 1. 10 X Blunting Kination Buffer 40 µl
 2. Blunting Kination Enzyme Mix 20 µl
 3. Ligation Mighty Mix 120 µl
 4. Control Vector (pUC118-*Hinc* II/BAP) (50 ng/µl) 20 µl
 5. Control Insert (200 ng/µl) 10 µl
 6. ddH₂O 340 µl
- Micropure®-EZ 24 sets, available from Millipore

The Reagent Set for Mighty Cloning Kit (Blunt End) (Code 6027) and Micropure®-EZ (Millipore) are sold separately.

Ordering information

Cat. #	Product	Size
R010A	PrimeSTAR® HS DNA Polymerase	250 Units
R010B	PrimeSTAR® HS DNA Polymerase	4 x 250 U
R040A	PrimeSTAR® HS (Premix)	5 × 500 µl 2X premix
R044A	PrimeSTAR® HS DNA Polymerase with GC Buffer	250 Units
R044B	PrimeSTAR® HS DNA Polymerase with GC Buffer	4 x 250 U
6027	Reagent Set for Mighty Cloning Kit	20 React  Manual  Cat info
TP600	Thermal Cycler Dice Gradient	1 Unit  BV article