Cat. # RR070A

For Research Use

TaKaRa

SpeedSTAR™ HS DNA Polymerase

Product Manual

v201906Da

Table of Contents

I. Description 3
II. Components 3
III. Storage 3
IV. Supplied Buffers 3
V. General Reaction Mixture 4
VI. Thermal Cycling Conditions 4
VII. Optimization of Parameters 4
VIII. Experimental Examples 5
IX. Amplified Products 7
X. Troubleshooting 8

I. Description

SpeedSTAR HS DNA Polymerase is designed for high speed PCR and includes two optimized buffers, Fast Buffer I and II. SpeedSTAR HS DNA Polymerase is compatible with an extension time as fast as 10 sec/kb, compared to general PCR enzymes that typically require 1 min/kb. SpeedSTAR HS DNA polymerase utilizes a monoclonal antibody-mediated hot start formulation, thereby preventing non-specific amplification due to mispriming and/or formation of primer dimers during reaction assembly. This enzyme can be used with routine PCR reaction conditions since the monoclonal antibody is denatured in the initial DNA-denaturation step.

II. Components (for 200 reactions ; 50 μl volume)

SpeedSTAR HS DNA Polymerase (5 units/μl)*1	50 μl
10X Fast Buffer I (Mg^{2+} plus)*2	1 ml
10X Fast Buffer II (Mg^{2+} plus)*2	1 ml
dNTP Mixture (2.5 mM each)	800 μl

*1 【Storage Buffer】

20 mM	Tris-HCl (pH 8.0)
100 mM	KCl
0.1 mM	EDTA
1 mM	DTT
0.5%	Tween 20
0.5%	NP-40
50%	Glycerol

【Unit definition】

One unit is the amount of enzyme that will incorporate10 nmol of dNTP into acid-insoluble products in 30 minutes at 74℃ with activated salmon sperm DNA as the template-primer.

*2 Mg^{2+} concentration: 10X Fast Buffer I, 30 mM ; 10X Fast Buffer II, 20 mM

III. Storage

-20℃

IV. Supplied Buffers

Two Fast Buffers are supplied with this product. Select the appropriate buffer depending on the target amplification size. For amplicons up to 2 kb, Fast Buffer I should be used. Either Fast Buffer I or II can be used for amplicons between 2 - 4 kb. For amplification of targets over 4 kb, Fast Buffer II is preferred. The use of Fast Buffer I for longer amplification or Fast Buffer II for shorter amplification can result in lower amplification efficiency.

V. General Reaction Mixture (for 50 μl reactions)

Reagent	Volume/Amount	Final Conc.
SpeedSTAR HS DNA Polymerase (5 units/μl)	0.25 μl	1.25 units/50 μl
dNTP Mixture (2.5 mM each)	4 μl	200 μM
Primer 1	10 - 50 pmol	0.2 - 1 μM
Primer 2	10 - 50 pmol	0.2 - 1 μM
Template	< 500 ng	
10X Fast Buffer I or II	5 μl	1X
Sterile purified water	up to 50 μl	

NOTE: The reaction mixture can be prepared at room temperature. Keep reaction components on ice while assembling the reaction mixture.

VI. Thermal Cycling Conditions

SpeedSTAR polymerase can be used for both 2 step and 3 step PCR. For fast PCR, try the 2 step PCR method first. When using short primers, the 3 step PCR method is recommended. For long amplification, we recommend using a longer extension time. In some cases, using SpeedSTAR polymerase for long amplifications may result in smearing of the amplified products.

(A) 2 step PCR

- Amplification up to 4 kb or 6 kb (with Fast Buffer I or II)

98℃ 5 sec
65℃ 10 sec (or up to 20 sec/kb)] 30 cycles

- Amplification of 6 kb or longer (with Fast Buffer II)

98℃ 5 sec
68℃ 10 sec (or up to 20 sec)/kb] 30 cycles

NOTE: Efficient amplification can be achieved by optimizing the temperature of each step, depending on the amplicon size.

(B) 3 step PCR (with Fast Buffer I or II)

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

NOTE: Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. We recommend 5 - 10 sec at 98℃ or 20 - 30 sec at 95℃.

VII. Optimization of Parameters

The following parameters should be optimized for best performance of SpeedSTAR HS DNA polymerase.

1) Enzyme amount:
Use 1.25 units for a 50 μl PCR. This amount may be adjusted slightly depending on the template amount or amplicon size. Excess enzyme can result in non-specific amplification causing smeared bands on a gel; too little enzyme can lower the amplification efficiency.

2) Template DNA:
Excess template can result in non-specific amplification or smeared bands. Refer to the following recommendations for the amount of template for a 50 μl reaction.

Human genomic DNA	5 - 500 ng
E. coli genomic DNA	50 pg - 100 ng
Plasmid DNA	10 pg - 1 ng

3) Concentration of dNTPs and Mg^{2+}:
The supplied Fast Buffer I includes Mg^{2+} at a final concentration of 3 mM, and Fast Buffer II includes Mg^{2+} at a final concentration of 2 mM. The best results will be obtained when dNTPs are used at a final concentration of 200 μM each.

4) Primers:
Design primers using a commercially available primer design software (e.g. OLIGO Primer Analysis Software, Molecular Biology Insights)

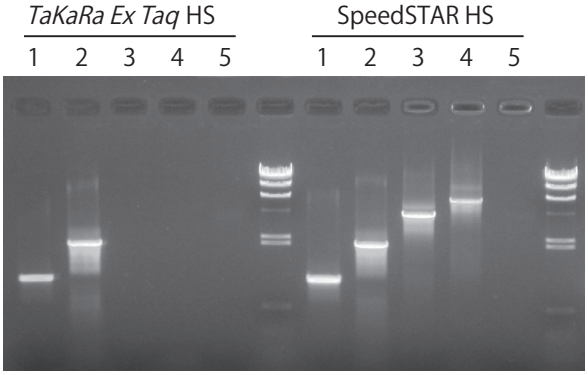
- Primer length
  In general, 20 - 25 -mer primers provide good results. For longer amplifications, 25 - 30 -mer primers may be better.
- GC content
  Primer GC content should be 40 - 60%. GC residues should be evenly distributed throughout each primer. The 3' termini should not be GC-rich.
- Tm value
  Tm values of the upstream and downstream primers should be the same.
- Primer concentration
  The concentration should be empirically determined within the range of 0.2 - 1.0 μM. Low primer concentration can result in lower yield of amplified products. In contrast, high primer concentration can cause non-specific amplification which can inhibit specific amplification.

VIII. Experimental Examples

< Fast PCR >

Comparison of SpeedSTAR HS DNA Polymerase and *TaKaRa Ex Taq*® Hot Start Version:
Amplification of products of various sizes was compared for the enzymes using 2 step PCR with extension time of 45 sec. Under these conditions, SpeedSTAR HS was able to amplify fragments up to 6 kb, while *TaKaRa Ex Taq* HS was only able to amplify products up to 2 kb.*

* *TaKaRa Ex Taq* HS can amplify 6 kb products when optimized conditions are used.



Template: *E.coli* genomic DNA 50 ng/50 μl
Amplified size:
1. 1 kb
2. 2 kb
3. 4 kb
4. 6 kb
5. 8 kb

Thermal cycling conditions

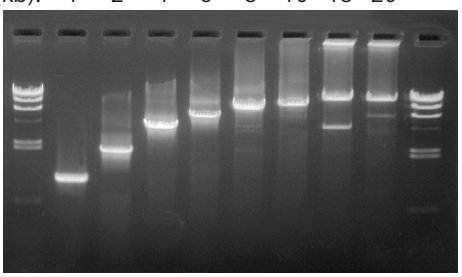
94℃	1 min	
↓		
95℃	5 sec	30 cycles
68℃	45 sec	

＜ High Performance ＞

Comparison of reaction time between SpeedSTAR polymerase and *TaKaRa Ex Taq* HS with varying amplification target sizes:

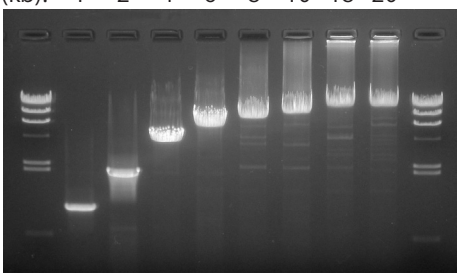
SpeedSTAR HS

Size (kb): 1 2 4 6 8 10 18 20



TaKaRa Ex Taq HS

Size (kb): 1 2 4 6 8 10 18 20



Thermal cycling conditions

Amplification of 1 kb, 2 kb (with Fast Buffer I)

94℃ 1 min
↓
95℃ 5 sec ┐
65℃ 20 sec ┘ 30 cycles

Total reaction time: approx. 33 min

Amplification of 4 kb, 6 kb (with Fast Buffer II)

94℃ 1 min
↓
95℃ 5 sec ┐
65℃ 60 sec ┘ 30 cycles

Total reaction time: approx. 53 min

Amplification of 8 kb, 10 kb (with Fast Buffer II)

94℃ 1 min
↓
98℃ 5 sec ┐
68℃ 2 min ┘ 30 cycles

Total reaction time: approx. 83 min

Amplification of 18 kb, 20 kb (with Fast Buffer II)

94℃ 1 min
↓
98℃ 5 sec ┐
68℃ 5 min ┘ 35 cycles
↓
72℃ 5 min

Total reaction time: approx. 3 hr 29 min

Amplification of 1 kb, 2 kb

94℃ 1 min
↓
98℃ 10 sec ┐
68℃ 2 min ┘ 30 cycles

Total reaction time: approx. 96 min

Amplification of 4 kb, 6 kb

94℃ 1 min
↓
98℃ 10 sec ┐
68℃ 6 min ┘ 30 cycles
↓
72℃ 10 min

Total reaction time: approx. 3 hr 46 min

Amplification of 8 kb, 10 kb

94℃ 1 min
↓
98℃ 10 sec ┐
68℃ 10 min ┘ 30 cycles
↓
72℃ 10 min

Total reaction time: approx. 5 hr 46 min

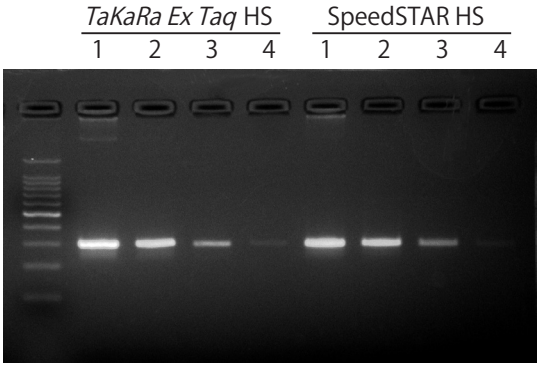
Amplification of 18 kb, 20 kb

94℃ 1 min
↓
98℃ 10 sec ┐
68℃ 15 min ┘ 30 cycles
↓
72℃ 10 min

Total reaction time: approx. 8 hr 16 min

< Fast PCR with high sensitivity and performance >

Comparison of detection sensitivity between SpeedSTAR polymerase and *TaKaRa Ex Taq* HS with varying amplification product sizes:



Amplified size: 300 bp
Template: human genomic DNA

1: 100 ng/50 μl PCR
2: 10 ng/50 μl PCR
3: 1 ng/50 μl PCR
4: 0.1 ng/50 μl PCR

Thermal cycling conditions

***TaKaRa Ex Taq* HS**

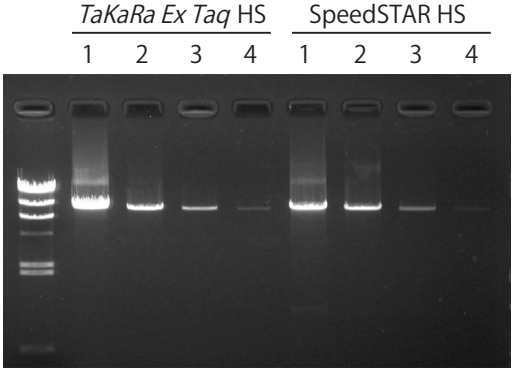
98℃ 5 sec
55℃ 30 sec
72℃ 30 sec
] 30 cycles

Total reaction time: approx. 67 min

SpeedSTAR HS (with Fast Buffer I)

98℃ 5 sec
55℃ 10 sec
72℃ 5 sec
] 30 cycles

Total reaction time: approx. 39 min



Amplified size: 8.5 kb
Template: human genomic DNA

1: 100 ng/50 μl PCR
2: 10 ng/50 μl PCR
3: 1 ng/50 μl PCR
4: 0.1 ng/50 μl PCR

Thermal cycling conditions

***TaKaRa Ex Taq* HS**

94℃ 1 min
↓
98℃ 5 sec
68℃ 8.5 min
] 30 cycles
↓
72℃ 10 min

Total reaction time: approx. 4 hr 59 min

SpeedSTAR HS (with Fast Buffer II)

94℃ 1 min
↓
98℃ 5 sec
68℃ 2 min
] 35 cycles
↓
72℃ 3 min

Total reaction time: approx. 1 hr 40 min

IX. Amplified Products

Most PCR products amplified with SpeedSTAR HS DNA Polymerase have a single A overhang at the 3'-termini. Therefore, PCR products can be directly used for cloning into a T-vector. It is also possible to clone the product using blunt-end vectors after blunting and phosphorylation of the ends.

X. Troubleshooting

Observation	Possible Cause	Solution
No amplification or low yield	Extension time	Set the extension time to 20 sec/kb.
	Annealing temperature	Lower the temperature in decrements of 2℃. Perform 3 step PCR.
	Template DNA	Repurify template DNA. For long amplification, use intact, undegraded DNA
	Primer	Re-design primers. Increase the primer amount.
Extra bands Smeared bands	Extension time	Excess extension time affects the reaction. Use the following guidelines: 2 step PCR: 10 - 20 sec/kb 3 step PCR: 5 - 10 sec/kb
	Annealing temperature	Raise the temperature in increments of 2℃. Perform 2 step PCR.
	Template DNA	Use an appropriate amount of DNA. Excess template DNA affects the reaction.
	Primer	Reduce the primer amount.