Takara SpeedSTAR™ HS DNA Polymerase

Product No. TAK RR070A and B

1.0 Intended Use

SpeedSTAR™ HS DNA Polymerase is a PCR enzyme, designed for high speed PCR in combination with two optimized buffers, Fast Buffer I and II. With SpeedSTAR™ HS DNA Polymerase it is possible to set the extension time as low as 10 sec/kb, compare to general PCR enzymes which requires 1 min for a 1kb extension. Use of this enzyme thus shortens the total reaction time.

2.0 Components

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

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

3.0 General Reaction Mix and Conditions

General PCR Reaction Mixture (50 µL)

	Volume/Amount	Final Concentration
SpeedSTAR™ HS DNA Polymerase (5 units/µl)	0.25 µl	1.25units/50 ul
dNTP Mixture (2.5 mM each)	4 µl	200 µM
Primer 1	10-50 pmol	0.2 µM – 1 µM
Primer 2	10-50 pmol	0.2 µM – 1 µM
Template	< 500 ng	
10 x Fast Buffer I or II	5 µl	1x
Sterilized distilled water	up to 50 µl	

Thermocycling Conditions

(A) 2 step PCR

Application up to 4 or 6 kbp (with Fast Buffer I or II)

95°C	5 sec.	30 cycles
65°C	10 (-20 sec.)/kb	

Application over 4 or 6 kbp (with Fast Buffer II)

98°C	5 sec.	30 cycles
68°C	10 (-20 sec.)/kb	

As shown above, efficient amplification can be achieved by setting the thermal cycling conditions depending on an amplifed size.

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

Application over 4 or 6 kbp (with Fast Buffer II)

98°C	5 sec.	30 cycles
55°C	10 ~ 15 sec.	
72°C	5 ~ 10 sec./kbp	

NOTE: The initial denaturation step is performed for 1 min. at 94°C.

*Refer to the following FAQs.

4.0 Frequently Asked Questions

4.1: What is the recommended enzyme concentration for SpeedSTAR™?

Generally, it is recommended to use 1.25 units for a 50 µl PCR. It may be adjusted slightly depending on a template amount or amplified size. Excess enzyme amount can result in non-specific amplification or smear. On the other hand, less enzyme concentration can lower the amplification efficiency.

4.2: What concentration of dNTP and Mg^{2+} are used in a SpeedSTAR™ reaction?

The supplied Fast Buffer I includes Mg^{2+} to have its final concentration of 3 mM , and Fast Buffer II of 2 mM in the final reaction mixture. The reaction mixture is designed to give the best result when the dNTP concentration is used at a final concentration of 200 µM each.

TaKaRa
Takara Mirus Bio

510 Charmany Drive • Madison, WI 53719 USA
Ph. 888-251-6618 or 608.441.2844 • Fax. 608.441-2845
www.takaramirusbio.com

rev. 0806

3.0 Frequently Asked Questions

4.3: I am observing smearing of my PCR product after agarose gel electrophoresis. What might be the problem?

Usually smearing of PCR product is observed when PCR conditions are not optimal. Try modifying your PCR cycling conditions using one or more of the following suggestions:

Extension time: Excess extension time affects the reaction. Refer to the following guideline.
2-step PCR: 10-20 sec /kb
3-step PCR: 5-10 sec /kb

Annealing temperature: Raise the temperature in increments of 2°C. Use 2-step PCR.

Template DNA: Use an appropriate amount of DNA. Excess template DNA affects the reaction.

Primer: Reduce the primer amount.

4.4: I observed little or no PCR product band on my agarose gel. How can I get more PCR product produced?

Usually little or no PCR product is observed when PCR conditions are not optimal. Try modifying your PCR cycling conditions using one or more of the following suggestions:

Extension time:	Set the extension time at 20 sec/kb
Annealing temperature:	Lower the temperature in decrements of 2°C. Use 3-step PCR.
Template DNA:	Repurify template DNA. For long amplifications, intact DNA with the least damage should be used.
Primer:	Redesign primers. Or increase the primer amount.

4.5: What is the recommended amount of template DNA needed in a SpeedSTAR™ reaction?

The proper amount of template DNA to be used in a SpeedSTAR™ reaction varies with the DNA source. Excess template can result in non-specific amplification or smear.
Refer to the following for the recommended amount of template for a 50 µl PCR.:

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

4.6: What type of PCR product ends does SpeedSTAR™ generate?

Most of the PCR products amplified with SpeedSTAR™ HS DNA Polymerase have one A added at 3'-termini, therefore PCR products can be directly used for cloning into T-vector. In addition, it is possible to clone the product in blunt-end vectors after blunting and phosphorylation of the end.

4.7: Are there any guidelines I should use when designing my primers for a PCR reaction using SpeedSTAR™?

Design the most appropriate sequence using a commercially available primer designing software (e.g. OLIGO™ Primer Analysis Software by MBI).

Primer length:	Basically 20 – 25-mer primers will provide good results. Sometimes in longer amplifications, the use of 25-30-mer primer is better.
GC content:	The content should be 40-60%. GC should locate uniformly in a primer, not in bias location. The 3′ termini of the primer should not be GC rich.
T_m value:	T_m values of upstream and downstream primers should be the same.
Primer concentration:	It should be empirically determined within the range of 0.2 µM – 1.0 µM. Lower primer concentration can result in lower amplified products. On the contrary, higher primer concentration can cause non-specific amplification which can inhibit the specific amplification.

TaKaRa
Takara Mirus Bio

510 Charmany Drive • Madison, WI 53719 USA
Ph. 888-251-6618 or 608.441.2844 • Fax. 608.441-2845
www.takaramirusbio.com