Takara PrimeSTAR™ HS DNA Polymerase

Product No. TAK R010A and B

1.0 Components

5X PCR Buffer	1 mL X 2
dNTP Mixture 2.5 mM each	800 µL
PrimeSTAR™ (2.5 U/µl)	100 µL

2.0 General Reaction Mix and Conditions

General PCR Reaction Mixture (50 µL)

5X PCR Buffer	10 µL
2.5 mM dNTP Mixture	4 µL
Primer 1	0.2 µM (final conc.)
Primer 2	0.2 µM (final conc.)
Template	500 ng
PrimeSTAR™ (2.5U/µL)	0.5 µL
Sterilized dH_2O	up to 50 µL

Thermocycling Conditions

98°C	10 sec.	30 cycles
55°C	**5 sec.** or **15 sec.***	
72°C	1 min/kb	
	OR	
98°C	10 sec.	30 cycles
68°C	1 min/kb	

NOTE: Takara does not perform an initial dentauration step, however, if it is necessary to perform this step, 94°C for 30 sec. is the proper setting.

3.0 Frequently Asked Questions

3.1: What are the PrimeSTAR™ recommended annealing conditions?

Takara recommends the following annealing conditions:

The initial annealing temperature should be 55°C as a starting point.
Annealing time: Because PrimeSTAR has very high priming efficiency, set the annealing time at 5 sec. or 15 sec., based upon T_m value. A longer annealing time can result in smearing.

When $T_{m*} > 55°C$: 5 sec.
When $T_{m*} < 55°C$: 15 sec.

Calculate the T_m value using the following method: *T_m Calculation formula: T_m (°C) = 2 (NA + NT) + 4 (NC + NG) -5
Use this method only for a primer <25 bases. For primers >25 bases, set the annealing temperature to 5 sec.

Note: Please follow the provided guidelines as directed.

3.2: When should I use a 3-Step vs. a 2-Step PCR cycling protocol?

Generally, a 3 step protocol is recommended. However, when smearing of product is observed following agarose gel electrophoresis, or when primers with a $T_m > 70°C$ are used, a 2 step protocol is recommended.

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3.0 Frequently Asked Questions

3.3: I am observing smearing of my PCR product after agarose gel electrophoresis. What is 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:

- Decrease the annealing time, e.g. if annealing is performed at 15 sec., then decrease the time to 5 sec.
- Raise the annealing temperature to 58-65°C.
- Switch from the 3-Step to the 2-Step cycling protocol.

3.4: The amount of obtained PCR product in my PrimeSTAR™ reaction was low and I observed little or no product on my agarose gel. How can I increase my yield?

Often a low amount of PCR product is the result of improper annealing of the primers to the DNA template. Try modifying your annealing conditions by either:

- Extending the annealing time, e.g. if annealing is performed for 5 sec., then extend the time to 15 sec., or lowering the annealing temperature to 50-53°C.

3.5: What is the recommended amount of template DNA needed in a PrimeSTAR™ reaction?

The proper amount of template DNA to be used in a PrimeSTAR™ reaction varies with the DNA source:

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

3.6 Can the concentration of dNTPs be modified?

Excess dNTPs have a chelative effect, higher dNTP concentrations will lower the effective Mg^{2+} concentration of the reaction mixture. The supplied 5X PrimeSTAR™ Buffer provides a 1 mM final Mg^{2+} reaction mix concentration that has been optimized for use with a 200 μM final dNTP concentration in the reaction mix. Thus, the concentration of dNTPs should not be modified.

3.7: What buffer should I use for agarose gel electrophoresis of my PrimeSTAR™ product?

TAE Buffer is recommended for agarose gel electrophoresis of amplified products that are obtained using PrimeSTAR™ HS DNA Polymerase. Use of TBE Buffer may result in DNA band patterns which are enlarged at the gel bottom.

3.8: What type of PCR product ends does PrimeSTAR™ generate?

All PCR products amplified with PrimeSTAR™ HS DNA Polymerase have blunt-end termini. Accordingly, they can directly be cloned into blunt-end vectors (if necessary, phosphorylate before cloning), but are not clonable into T-vectors.

3.9: Do PrimeSTAR™ products require any special treatment before doing a restriction digest?

Prior to performing restriction enzyme digestion of amplified PCR products, remove all traces of PrimeSTAR™ HS polymerase from the reaction mix by phenol/chloroform extraction. Particularly for 3'-protruding restriction enzymes, such as Pst I, the 3'-protruding termini produced by these enzymes may be deleted during digestion by 3'–5' exonuclease activity of residual amounts of PrimeSTAR™ HS polymerase.

3.10: Can I use the PrimeSTAR™ products directly in a sequencing reaction?

Takara recommends that PCR products be extracted with phenol/chloroform prior to direct sequencing to ensure inactivation of any remaining 3'–5' exonuclease polymerase activity.