Thermo Scientific Phire Hot Start II DNA Polymerase

F-122S, 200 reactions 50 µl each (200 µl)
F-122L, 1000 reactions 50 µl each (1.0 ml)

Store at -20°C

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1. Introduction

Thermo Scientific Phire Hot Start II DNA Polymerase is a novel DNA polymerase designed for use in all routine and high throughput PCR applications. A special DNA-binding domain linked to the Phire® Hot Start II DNA Polymerase enhances the processivity of the polymerase, enabling short extension times and improved yields. The polymerase is also capable of amplifying long DNA fragments, such as the 7.5 kb genomic DNA used in Thermo Scientific quality control assays. Phire Hot Start II DNA Polymerase provides 2-fold fidelity compared to *Taq* DNA polymerase. The hot start modification of the polymerase is based on the Affibody inactivation method.[1,2] It inhibits DNA polymerase activity at ambient temperatures, thus preventing amplification of non-specific products. At polymerization temperatures the Affibody® molecule is released, rendering the polymerase fully active. Phire Hot Start II DNA Polymerase generates blunt ends in the amplification products. It does not possess the 5'→3' exonuclease activity needed for hydrolysis experiments.

IMPORTANT NOTES

- Use 98°C for denaturation. (See 5.1 & 5.2)
- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Section 5.3 carefully.
- Use 0.4 µl of enzyme per 20 µl reaction and 1 µl per 50 µl reaction.
- Use 200 µM of each dNTP. Do not use dUTP. (See 4.3)
- Use 10–15 s/kb for extension. (See 5.4)
- Note: Phire Hot Start DNA Polymerase produces blunt end

2. Package information

F-122S	200 reactions Material provided: Phire Hot Start II DNA Polymerase 200 µl (200 rxns in 50 µl), 5x Phire Reaction Buffer (2 x 1.5 ml) and DMSO (500 µl)
F-122L	1000 reactions Material provided: Phire Hot Start II DNA Polymerase 1.0 ml (1000 rxns in 50 µl), 5x Phire Reaction Buffer (7 x 1.5 ml) and DMSO (500 µl)

Material safety datasheet (MSDS) is available at www.thermoscientific.com/fzmsds.

3. Guidelines for using Phire Hot Start II DNA Polymerase

Phire Hot Start II DNA Polymerase is provided with 5x Phire Reaction Buffer. The buffer contains 1.5 mM $MgCl_2$ at final reaction concentration. A separate tube of DMSO is provided for further optimization.

3.1 Basic reaction conditions for PCR

Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. When using Phire Hot Start II DNA Polymerase, the PCR setup can be performed at room temperature. Prepare a master mix for the appropriate number of samples to be amplified. The DNA polymerase should be pipetted carefully and gently as the high glycerol content (50 %) in the storage buffer may otherwise lead to pipetting errors.

Table 1. Pipetting instructions (add items in this order).

Component	50 µl reaction	20 µl reaction	Final conc.
H_2O	add to 50 µl	add to 20 µl	
5x Phire Reaction Buffer	10 µl	4 µl	1x
10 mM dNTPs	1 µl	0.4 µl	200 µM each
Primer A (see 4.2)	x µl	x µl	0.5 µM
Primer B (see 4.2)	x µl	x µl	0.5 µM
Template DNA (see 4.4)	x µl	x µl	
Phire Hot Start II DNA Polymerase	1 µl	0.4 µl	

Table 2. Cycling instructions.

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	30 s	98°C	30 s	1
Denaturation Annealing (see 5.3) Extension	98°C – 72°C	5 s – 10 –15 s/1 kb	98°C X°C 72°C	5 s 5 s 10–15 s/1 kb	25–35
Final extension	72°C 4°C	1 min hold	72°C 4°C	1 min hold	1

4. Notes about reaction components

4.1 Enzyme

The optimal amount of enzyme is 0.4 µl in 20 µl reaction and 1 µl in 50 µl reaction. When cloning fragments amplified with Phire Hot Start II DNA Polymerase blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with Thermo Scientific Taq DNA Polymerase, for example. A protocol for TA cloning of PCR fragments amplified with Phire Hot Start II DNA Polymerase can be found on website www.thermoscientific.com/pcrcloning.

4.2 Primers

The recommendation for final primer concentration is 0.5 µM. If required, the primer concentration may be optimized between 0.2–1.0 µM. The results from primer Tm calculations can vary significantly depending on the method used. Always use the Tm calculator and instructions on website www.thermoscientific.com/pcrwebtools to determine the Tm values of primers and optimal annealing temperature. If using a two-step PCR protocol, where both primer annealing and extension occur in a single step at 72°C, the primers should be designed accordingly.

4.3 Mg^{2+} and dNTP

The concentration of Mg^{2+} in Phire Reaction Buffer is optimized to work well for most amplicons. High quality dNTPs should be used for optimal performance with Phire Hot Start II DNA Polymerase. The polymerase cannot read dUTP-derivatives or dITP in the template strand so the use of these analogues or primers containing them is not recommended. For optimal results always use 200 µM of each dNTP.

4.4 Template

General guidelines for low complexity DNA (e.g. plasmid, lambda or BAC DNA) are: 1 pg–10 ng per 20 µl reaction volume, or 2.5 pg– 25 ng per 50 µl reaction volume. For high complexity genomic DNA, the amount of DNA template should be 10–100 ng per 20 µl reaction volume, or 25–250 ng per 50 µl reaction volume. If cDNA synthesis reaction mixture is used directly as a source for the template, the volume used should not exceed 10 % of the final PCR reaction volume.

4.5 PCR additives

The recommended reaction conditions for GC-rich templates include 3 % DMSO as a PCR additive, which aids in the denaturing of templates with high GC contents. For further optimization the amount of DMSO should be increased in 2 % increments. In some cases DMSO may also be required for supercoiled plasmids to relax for denaturation. Other PCR additives such as formamide, glycerol, and betaine are also compatible with Phire Hot Start II DNA Polymerase. If high DMSO concentration is used, the annealing temperature must be decreased, as DMSO affects the melting point of the primers. It has been reported that 10 % DMSO decreases annealing temperature by 5.5–6.0°C[3].

5. Notes about cycling conditions

Due to the nature of Phire Hot Start II DNA Polymerase, optimal reaction conditions may differ from other amplification protocols. Please pay special attention to the conditions listed below when running your reactions. Following the guidelines will ensure optimal enzyme performance.

5.1 Initial denaturation

Denaturation should be performed at 98°C. Due to the high thermostability of Phire Hot Start II DNA Polymerase, even higher than 98°C temperatures may be used. We recommend a 30-second initial denaturation at 98°C for most templates. Some templates may require longer initial denaturation time and the length of the initial denaturation time can be extended up to 3 minutes. Because of the unique hot start technology utilized, Phire Hot Start II DNA Polymerase does not require a separate enzyme reactivation step.

5.2 Denaturation

Keep the denaturation time as short as possible. Usually 5 seconds at 98°C is enough for most templates. **Note:** The denaturation time and temperature may vary depending on the ramp rate and temperature control mode of the cycler.

5.3 Primer annealing

As a basic rule, for primers >20 nt, anneal for 5 seconds at a Tm +3°C of the lower Tm primer. For primers ≤ 20 nt, use an annealing temperature equal to the Tm of the lower Tm primer. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR). Two-step cycling without an annealing step is recommended for high Tm primer pairs. Use the Tm calculator and instructions on website www.thermoscientific.com/pcrwebtools to determine primer Tm and optimal annealing temperature.

5.4 Extension

The extension should be performed at 72°C. Extension time of 10 seconds per 1 kb is recommended for most templates. However, higher yields may be obtained using extension time of 15 s/kb for some challenging primer-template pairs.

6. Troubleshooting

No product at all or low yield	
• Optimize annealing temperature. • Repeat and make sure that there are no pipetting errors. • Make sure the cycling protocol was performed as recommended. • Use fresh high-quality dNTPs. • Do not use dNTP mix or primers that contain dUTP or dITP. • Titrate template amount. • Template DNA may be damaged. Use carefully purified template.	• Increase extension time. • Increase the number of cycles. • Titrate DMSO (2–8 %) in the reaction (see section 4.5). • Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 98°C or higher. • Optimize denaturation time. • Check the purity and concentration of the primers. • Check primer design.
Non-specific products - High molecular weight smears	
• Make sure the extension time used was not too long. • Reduce the total number of cycles. • Increase annealing temperature or try 2-step protocol (see section 5.3).	• Titrate template amount • Optimize denaturation temperature (see section 5.2). • Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• Increase annealing temperature (see section 5.3). • Make sure the extension time used was not too long.	• Titrate template amount. • Decrease primer concentration. • Design new primers.

7. Component specifications

7.1 Phire Hot Start II DNA Polymerase (F-122)

Thermostable Phire DNA Polymerase is isolated and purified from an *E. coli* strain carrying a plasmid with the cloned archaeal DNA polymerase gene. Phire Hot Start II DNA Polymerase possesses the following activities: 5´→3´ DNA polymerase activity and a weak 3´→5´ exonuclease activity. The Affibody ligand is purified from an *E. coli* strain carrying a plasmid encoding Affibody protein. Phire Hot Start II DNA Polymerase is free of contaminating endo- and exonucleases.

Storage buffer: 20 mM Tris-HCl (pH 7.4 at 25°C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 200 µg/ml BSA and 50 % glycerol.
DNA amplification assay: Performance in PCR is tested by the amplification of 500 bp and 7.5 kb fragments of genomic DNA.
Exonuclease contamination assay: Incubation of 10 U for 4 hours at 72°C in 50 µl assay buffer with 1 µg sonicated [^{3}H] ssDNA (2x10^5 cpm/µg) released < 1 % of radioactivity.
Endonuclease contamination assay: No endonuclease activity was observed after incubation of 10 U of DNA polymerase with 1 µg of λ DNA in assay buffer at 72°C for 4 hours.

7.2 5x Phire Reaction Buffer (F-524)

The 5x Phire Reaction Buffer contains 7.5 mM $MgCl_2$, which provides 1.5 mM $MgCl_2$ in final reaction conditions.

7.3 Dimethyl sulfoxide DMSO, 100 % (F-515)

Note: The freezing point of DMSO is 18–19°C, so it does not melt on ice.

8. References

1. Nord K. *et al.* (1997) *Nature Biotechnol.* 15: 772–777.
2. Wikman M. *et al.* (2004) *Protein Eng., Des. Sel.* 17: 455–462.
3. Chester N. & Marshak D.R. (1993) *Analytical Biochemistry.* 209: 284–290.

Shipping and storage

Phire Hot Start II DNA Polymerase is shipped on gel ice. Upon arrival, store the components at -20°C.

Technical support:

US: techservice.genomics@thermofisher.com
Europe, Asia, Rest of World:
techservice.emea.genomics@thermofisher.com

Web: www.thermoscientific.com/phire
Tm-calculator: www.thermoscientific.com/pcrwebtools

Product use limitation

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