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PRODUCT INFORMATION

Thermo Scientific
Phire Green Hot Start II PCR Master Mix

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Lot _ Expiry Date _

Store at -20 °C

Ordering information

Component	#F-126S 200 rxns 200 × 50 µL rxns	#F-126L 1000 rxns 1000 × 50 µL rxns
2X Phire Green Hot Start II PCR Master Mix	4 × 1.25 mL	20 × 1.25 mL
100% DMSO	1 × 0.5 mL	4 × 0.5 mL
Water, nuclease free	4 × 1.25 mL	20 × 1.25 mL

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1.Introduction

Thermo Scientific™ Phire™ Hot Start II DNA Polymerase is a unique DNA polymerase designed for use in all routine and high throughput PCR applications. A special DNA-binding domain linked to the Phire 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.

Phire Green Hot Start II PCR Master Mix is a convenient 2X mix containing Phire Hot Start II DNA Polymerase, nucleotides and optimized reaction buffer including $MgCl_2$. Only template and primers need to be added by the user.

The master mix also includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The dyes do not interfere with PCR performance and are compatible with downstream applications such as DNA sequencing, ligation and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless Phire Hot Start II PCR Master Mix (#F-125S) or purifying the PCR product prior to analysis.

2. 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 before setting annealing temperature.
- Use 10–15 s/kb for extension. (See 5.4)
- Phire Hot Start II DNA Polymerase produces blunt end DNA products.

3. Setting up PCR reactions using Phire Hot Start II PCR Master Mix

Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. When using Phire Green Hot Start II PCR Master Mix, it is not necessary to perform the PCR setup on ice.

Due to the unique nature of Phire Hot Start II DNA Polymerase, optimal reaction conditions may differ from standard enzyme protocols. Phire Hot Start II DNA Polymerase tends to work better at elevated denaturation and annealing temperatures due to higher salt concentrations in its buffer. Please pay special attention to the conditions listed in section 5 when running your reactions. Following the guidelines will ensure optimal enzyme performance.

Table 1. Pipetting instructions: add items in this order.

Component	20 µL rxn	50 µL rxn	Final conc.
H_2O	add to 20 µL	add to 50 µL	
2X Phire Green HS II Master Mix	10 µL	25 µL	1X
Forward primer *	X µL	X µL	0.5 µM
Reverse primer *	X µL	X µL	0.5 µM
Template DNA	X µL	X µL	
(DMSO, optional)**	(0.6 µL)	(1.5 µL)	(3%)

* The recommendation for final primer concentration is 0.5 µM, but it can be varied in a range of 0.2–1.0 µM, if needed.

** Addition of DMSO is recommended for GC-rich amplicons. DMSO is not recommended for amplicons with very low GC %.

Table 2. Cycling instruction

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 (see 5.4)	98 °C – 72 °C	5 s – 10–15 s/kb	98 °C X °C 72 °C	5 s 5 s 10–15 s/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. 2X Phire Green Hot Start II PCR Master Mix

Phire Green Hot Start II PCR Master Mix contains all the necessary reaction components except for template DNA and primers. The master mix provides 1.5 mM $MgCl_2$ and 200 µM of each dNTP in final reaction concentration. Phire Hot Start II DNA Polymerase concentration is optimized to give good results in most reactions.

4.2. Template

For low complexity DNA (e.g. plasmid, lambda or BAC DNA) it is recommended to use 2.5 pg–25 ng per 50 µL reaction volume. For high complexity genomic DNA, the amount of DNA template should be 25–250 ng per 50 µL reaction volume.

If cDNA synthesis reaction mixture is used as a source of template, the volume of the template should not exceed 10% of the final PCR reaction volume.

4.4. 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 DMSO should be varied in 2% increments. In some cases DMSO may also be required for supercoiled plasmids to relax for denaturation. 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 the annealing temperature by 5.5–6.0 °C.[3]

5. Notes about cycling conditions

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 denaturation temperatures can be used. We recommend 30 seconds 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.

5.2. Denaturation

Keep the denaturation 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

The optimal annealing temperature for Phire Hot Start II DNA Polymerase may be significantly different than annealing temperature with other DNA polymerases. Always use the Tm calculator and instructions on our website (www.thermofisher.com/tmcalculator) to determine the Tm values of your primers and optimal annealing temperature.

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 annealing step is also recommended for high Tm primer pairs.

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 with challenging primer-template pairs.

6. Cloning recommendations

Blunt end cloning is recommended when cloning DNA fragments amplified with Phire Green Hot Start II PCR Master Mix. If TA cloning is required, it is necessary to add A overhangs to the PCR product (with Thermo Scientific *Taq* DNA Polymerase, for example). A detailed protocol for TA cloning of fragments amplified with Phire Green Hot Start II PCR Master Mix can be found on our website www.thermofisher.com.

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7. Troubleshooting

No product at all or low yield	
• Repeat and make sure that there are no pipetting errors. • Titrate template amount. • Template DNA may be damaged. Use carefully purified template. • Increase extension time. • Increase the number of cycles. • Optimize annealing temperature.	• Titrate DMSO (2–8%) in the reaction. • 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	
• Shorten extension time. • Reduce the total number of cycles. • Increase annealing temperature or try 2-step protocol.	• Vary denaturation temperature • Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• Increase annealing temperature • Shorten extension time. • Titrate template amount.	• Decrease primer concentration. • Design new primers.

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.

CERTIFICATE OF ANALYSIS

DNA amplification assay
Performance in PCR is tested by the amplification of a 7.5 kb fragment of genomic DNA.

Quality authorized by:  Jurgita Zilinskiene