FINNZYMES

Phusion®

NEW ENGLAND BioLabs Inc.
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High-Fidelity PCR Master Mix

Product codes:
F-531S: Phusion® Master Mix with HF Buffer, 100 reactions
F-531L: Phusion® Master Mix with HF Buffer, 500 reactions
F-532S: Phusion® Master Mix with GC Buffer, 100 reactions
F-532L: Phusion® Master Mix with GC Buffer, 500 reactions

Stable for one year from the packaging date. Store at -20°C.

1. Introduction

Finnzymes' Phusion® High-Fidelity DNA Polymerase offers extreme performance for all PCR applications. Incorporating an exciting new technology, Phusion DNA Polymerase brings together a novel *Pyrococcus*-like enzyme with a processivity-enhancing domain. The Phusion DNA Polymerase generates long templates with an accuracy and speed previously unattainable with a single enzyme, even on the most difficult templates. The extreme fidelity makes Phusion DNA Polymerase a superior choice for cloning. Using a *lac*I-based method modified from previous studies,[1] the error rate of Phusion DNA Polymerase is determined to be 4.4 x 10^{-7} in Phusion HF Buffer, which is approximately 50-fold lower than that of *Thermus aquaticus* DNA polymerase, and 6-fold lower than that of *Pyrococcus furiosus* DNA polymerase.

Phusion DNA Polymerase possesses the following activities: 5´→3´ DNA polymerase activity and 3´→5´ exonuclease activity. It generates blunt ends in the amplification products.

Phusion High-Fidelity PCR Master Mix is a convenient 2x mix containing Phusion DNA Polymerase, nucleotides and optimized reaction buffer including $MgCl_2$. Only template and primers need to be added by the user.

Important notes

- Use 98°C for denaturation. (See 5.1 & 5.2)
- Anneal at Tm+3°C (> 20nt) or use 2-step protocol. (See 5.3)
- Use 15–30 sec/kb for extension. Do not exceed 1 min/kb. (See 5.4)
- Note: Phusion DNA Polymerase produces blunt end PCR products.

2. Package information

F-531S	100 reactions in 50 µl volume **2x Phusion® Master Mix with HF Buffer** (2 x 1.25 ml) Contains: 0.04 U/µl Phusion® DNA Polymerase 2x Phusion® HF Buffer* 400 µM of each dNTP **100 % DMSO** (500 µl)
F-531L	500 reactions in 50 µl volume **2x Phusion® Master Mix with HF Buffer** (10 x 1.25 ml) Contains: 0.04 U/µl Phusion® DNA Polymerase 2x Phusion® HF Buffer* 400 µM of each dNTP **100 % DMSO** (2 x 500 µl)
F-532S	100 reactions in 50 µl volume **2x Phusion® Master Mix with GC Buffer** (2 x 1.25 ml) Contains: 0.04 U/µl Phusion® DNA Polymerase 2x Phusion® GC Buffer* 400 µM of each dNTP **100 % DMSO** (500 µl)
F-532L	500 reactions in 50 µl volume **2x Phusion® Master Mix with GC Buffer** (10 x 1.25 ml) Contains: 0.04 U/µl Phusion® DNA Polymerase 2x Phusion® GC Buffer* 400 µM of each dNTP **100 % DMSO** (2 x 500 µl)

* Both 2x Phusion HF Buffer and 2x Phusion GC Buffer provide 1.5 mM $MgCl_2$ in final reaction concentration.

Materia safety data sheet (MSDS) is available at www.finnzymes.fi.

3. Setting up PCR reactions using Phusion® PCR Master Mix

Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. PCR reactions should be set up on ice.

Due to the novel nature of Phusion DNA Polymerase, optimal reaction conditions may differ from standard enzyme protocols. Phusion 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	50 µl reaction	20 µl reaction	Final conc.
H_2O	add to 50 µl	add to 20 µl	
2x Phusion® Master Mix	25 µl	10 µl	1x
primer A*	x µl	x µl	0.5 µM
primer B*	x µl	x µl	0.5 µM
template DNA	x µl	x µl	
(DMSO**, optional)	(1.5 µl)	(0.6 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% or amplicons that are >20kb.

Table 2. Cycling instructions.

	2-step protocol		3-step protocol		
Cycle step	Temp.	Time	Temp.	Time	Cycles
Initial denaturation	98°C	30 s	98°C	30 s	1
Denaturation Annealing (see 5.3) Extension (see 5.4)	98°C – 72°C	5–10 s – 15–30 s /1 kb	98°C X°C 72°C	5–10 s 10–30 s 15–30 s /1 kb	25–35
Final extension	72°C 4°C	5–10 min hold	72°C 4°C	5–10 min hold	1

4. Notes about reaction components

4.1 Enzyme

In Phusion PCR Master Mix the enzyme concentration is optimized to give good results in most reactions. When pipetted according to the instructions the final concentration is 1 U of enzyme in 50 µl reaction (0.4 U in 20 µl reaction).

When cloning fragments amplified with Phusion 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 DyNAzyme™ II DNA Polymerase (F-501), for example. However, before adding the overhangs it is very important to remove all the Phusion DNA Polymerase by purifying the PCR product carefully. Any remaining Phusion DNA Polymerase will degrade the A overhangs, creating blunt ends again. A detailed protocol for TA cloning of fragments amplified with any of the Phusion DNA polymerases can be found on Finnzymes website (www.finnzymes.com).

4.2 Buffers

The F-531 Phusion PCR Master Mix contains Phusion HF Buffer. The F-532 Phusion PCR Master Mix contains Phusion GC Buffer. The error rate of Phusion DNA Polymerase in HF Buffer (4.4×10^{-7}) is even lower than that in GC Buffer (9.5×10^{-7}). Therefore, the Master Mix with HF Buffer should be used as a default for high-fidelity amplification. However, GC Buffer can improve the performance of Phusion DNA Polymerase on some difficult or long templates, i.e. GC-rich templates or those with complex secondary structures.

4.3 Mg^{2+} and dNTP

The Phusion Master Mix provides 1.5 mM $MgCl_2$ and 200 µM of each dNTP in final reaction concentration.

4.4 Template

General guidelines for low complexity DNA (e.g. plasmid, lambda or BAC DNA) are: 1 pg–10 ng per 50 µl reaction volume. For high complexity genomic DNA, the amount of DNA template should be 50–250 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.

4.5 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 DMSO should be varied 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 Phusion PCR Master Mix. 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.[2]

5. Notes about cycling conditions

5.1 Initial denaturation

Denaturation should be performed at 98°C. Due to the high thermostability of Phusion 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 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 5–10 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 Phusion DNA Polymerase has the ability to stabilize primer-template hybridization. As a basic rule, for primers > 20nt, anneal for 10–30 seconds at a Tm +3°C of the lower Tm primer. The Tm's should be calculated with the nearest-neighbor method[3] as results from primer Tm calculations can vary significantly depending on the method used. 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. Instructions for Tm calculation and a link to a calculator using the nearest-neighbor method can be found on Finnzymes website (www.finnzymes.com).

5.4 Extension

The extension should be performed at 72°C. Extension time depends on amplicon length and complexity. For low complexity DNA (e.g. plasmid, lambda or BAC DNA) use extension time 15 s per 1 kb. For high complexity genomic DNA 30 s per 1 kb is recommended.

6. 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.

7. Component specifications

7.1 Phusion® High-Fidelity PCR Master Mix

2x Phusion PCR Master Mix contains 0.04 U/µl Phusion High-Fidelity DNA Polymerase, 2x Phusion HF Buffer (in F-531) or 2x Phusion GC Buffer (in F-532), and 400 µM of each dNTP.

Thermostable Phusion DNA Polymerase is isolated and purified from an *E.coli* strain carrying a plasmid with the cloned Phusion DNA Polymerase gene. Phusion DNA Polymerase is purified free of contaminating endo- and exonucleases.

DNA amplification test

Performance in PCR is tested by the amplification of 7.5 kb genomic DNA and 20 kb lambda DNA.

7.2 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. Frey M. & Suppmann B. (1995) *Biochemica* 2: 34–35.
2. Chester N. & Marshak D.R. (1993) *Analytical Biochemistry* 209: 284–290.
3. Breslauer K.J. *et al.* (1986) *PNAS* 83: 3746–3750.

Shipping and storage

Phusion PCR Master Mix is shipped on gel ice. Upon arrival, store the components at -20°C. Phusion PCR Master Mix is stable for six months from the packaging date when stored and handled properly. After thawing the mix can be refrozen or optionally stored at +4°C for three months.

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