Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific
Phusion High-Fidelity DNA Polymerase

#F-530S 100 U

Lot __ Expiry Date __

Store at -20°C

www.thermoscientific.com/onebio

Ordering information

Component	#F-530S 100 U	#F-530L 500 U
Phusion DNA Polymerase, 2 U/µL	50 µL	250 µL
5X Phusion HF Buffer*	2 × 1.5 mL	6 × 1.5 mL
5X Phusion GC Buffer*	1.5 mL	2 × 1.5 mL
50 mM $MgCl_2$ solution	1.5 mL	2 × 1.5 mL
DMSO	500 µL	500 µL

* Both 5X Phusion HF Buffer and 5X Phusion GC Buffer provide 1.5 mM $MgCl_2$ in final reaction concentration.

Rev.2

1.Introduction

Thermo Scientific Phusion High-Fidelity DNA Polymerase offers extreme performance for all major PCR applications. Incorporating an exciting 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 *lacI*-based method modified from previous studies[1], the error rate of Phusion DNA Polymerase in Phusion HF Buffer is determined to be 4.4×10^{-7}, 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. The polymerase is also capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb λ DNA used in Thermo Fisher Scientific quality control assays.

2. Important Notes

- Use 98°C for denaturation (see sections 5.1 and 5.2).
- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read section 5.3 carefully.
- Use 15–30 s/kb for extension. Do not exceed 1 min/kb (see section 5.4).
- Use Phusion DNA Polymerase at 0.5–1.0 U per 50 µL reaction volume. Do not exceed 2 U/50 µL (see section 4.1).
- Use 200 µM of each dNTP. Do not use dUTP (see section 4.3).
- Phusion DNA Polymerases produce blunt end DNA products.

3. Guidelines for using Phusion DNA Polymerase

Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. PCR reactions should be set up on ice. Prepare a master mix for the appropriate number of samples to be amplified. Phusion DNA Polymerase should be pipetted carefully and gently as the high glycerol content (50%) in the storage buffer may otherwise lead to pipetting errors. It is critical that the Phusion DNA Polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3´→5´ exonuclease activity that can degrade primers in the absence of dNTPs.

Due to the nature of Phusion DNA Polymerase, the optimal reaction conditions may differ from PCR protocols for standard DNA polymerases. Due to the high salt concentration in the reaction buffer, Phusion DNA Polymerase tends to work better at elevated denaturation and annealing temperatures. 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	
5X Phusion HF Buffer*	4 µL	10 µL	1X
10 mM dNTPs	0.4 µL	1 µL	200 µM each
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%)
Phusion DNA Polymerase	0.2 µL	0.5 µL	0.02 U/µL

* Optionally 5X Phusion GC Buffer can be used. See section 4.2 for details.
** 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 > 20 kb.

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	5–10 s – 15–30 s/kb	98°C X°C 72°C	5–10 s 10–30 s 15–30 s/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

The optimal amount of enzyme depends on the amount of template and the length of the PCR product. Usually 1 unit of Phusion DNA Polymerase per 50 µL reaction volume gives good results, but the optimal amount can range from 0.5 to 2 units per 50 µL reaction depending on amplicon length and difficulty. It is not recommended to exceed 2 U/50 µL (0.04 U/µL), especially for amplicons that are > 5kb.

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 Thermo Scientific *Taq* or DreamTaq DNA Polymerase, 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 DNA fragments amplified with any of the Phusion DNA polymerases can be found on our website (www.thermoscientific.com/pcrcloning).

4.2 Buffers

Two buffers are provided with the enzyme: 5X Phusion HF Buffer and 5X Phusion GC Buffer. The error rate of Phusion DNA Polymerase in HF Buffer (4.4×10^{-7}) is lower than that in GC Buffer (9.5×10^{-7}). Therefore, the HF Buffer should be used as the default buffer for high-fidelity amplification. However, GC Buffer can improve the performance of Phusion DNA Polymerase on some difficult or long templates, such as GC-rich templates or those with complex secondary structures. For applications such as microarray or DHPLC, where the DNA templates need to be free of detergents, detergent-free reaction buffers are available for Phusion DNA Polymerases.

4.3 Mg^{2+} and dNTP

The concentration of Mg^{2+} is critical since Phusion DNA Polymerase is a magnesium dependent enzyme. Excessive Mg^{2+} stabilizes the DNA double strand and prevents complete denaturation of DNA. Excess Mg^{2+} can also stabilize spurious annealing of primers to incorrect template sites and decrease specificity. Conversely, inadequate Mg^{2+} may lead to lower product yield. The optimal Mg^{2+} concentration also depends on the dNTP concentration, the specific template DNA and the sample buffer composition. In general, the optimal Mg^{2+} concentration is 0.5 to 1 mM over the total dNTP concentration for standard PCR. If the primers and/or template contain chelators such as EDTA or EGTA, the apparent Mg^{2+} optimum may be shifted to higher concentrations. If further optimization is needed, increase Mg^{2+} concentration in 0.2 mM steps.
High quality dNTPs should be used for optimal performance with Phusion 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. Due to the high processivity of Phusion DNA Polymerase there is no advantage of increasing dNTP concentrations. For optimal results always use 200 µM of each dNTP.

(continued on reverse page)

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 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 Phusion 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 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 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.

5.2 Denaturation

Keep the denaturation time 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 optimal annealing temperature for Phusion DNA Polymerase may differ significantly from that of *Taq*-based polymerases. Always use the Tm calculator and instructions on our website (www.thermoscientific.com/pcrwebtools) to determine the Tm values of primers and optimal annealing temperature.
The Phusion DNA Polymerase has the ability to stabilize primer-template hybridization. As a basic rule, for primers > 20 nt, anneal for 10–30 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).

A 2-step protocol is recommended when primer Tm values are at least 69°C (> 20 nt) or 72°C (≤ 20 nt) when calculated with our Tm calculator. In the 2-step protocol the combined annealing/extension step should be performed at 72°C even when the primer Tm is > 72°C.

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 an extension time of 15 seconds per 1 kb. For high complexity genomic DNA 30 seconds per 1 kb is recommended. For some cDNA templates, the extension time can be increased up to 40 seconds per 1 kb to obtain optimal results.

6. Troubleshooting

No product at all or low yield	
• Repeat and make sure that there are no pipetting errors. • Use fresh high quality dNTPs. • Do not use dNTP mix or primers that contain dUTP or dITP. • Sample concentration may be too low. Use more template. • Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented. • Increase extension time. • Increase the number of cycles. • Optimize annealing temperature.	• Optimize enzyme concentration. • 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 the denaturation time. • Check the purity and concentration of the primers. • Check primer design. • Try using the alternative GC Buffer (see section 4.2).
Non-specific products - High molecular weight smears	
• Decrease enzyme concentration (see section 4.1). • Decrease extension time (see section 5.4). • Reduce the total number of cycles. • Increase annealing temperature or try 2-step protocol (see section 5.3)	• Vary denaturation temperature (see section 5.2). • Optimize Mg^{2+} concentration. • Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• Increase annealing temperature (see section 5.3). • Decrease extension time (see section 5.4). • Decrease enzyme concentration.	• Optimize Mg^{2+} concentration. • Titrate template amount. • Decrease primer concentration. • Design new primers.

7. Component specifications

7.1 Phusion High-Fidelity DNA Polymerase (F-530)

Thermostable Phusion DNA Polymerase is purified from an *E.coli* strain expressing the cloned Phusion DNA Polymerase gene. Phusion DNA Polymerase possesses the following activities: 5´→3´ DNA polymerase activity and 3´→5´ exonuclease activity.
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.
Unit definition: One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTPs into a polynucleotide fraction at 74°C in 30 min.
Enzyme activity is assayed in the following mixture: 25 mM TAPS-HCl, pH 9.3 (at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.75 mM activated salmon milt DNA, 100 µM dCTP, 200 µM each dATP, dGTP, dTTP, 0.4 MBq/ml [^{3}H] dCTP.

7.2 5X Phusion HF Buffer (F-518)

The 5X Phusion HF Buffer contains 7.5 mM $MgCl_2$, which provides 1.5 mM $MgCl_2$ in final reaction conditions.

7.3 5X Phusion GC Buffer (F-519)

The 5X Phusion GC Buffer contains 7.5 mM $MgCl_2$, which provides 1.5 mM $MgCl_2$ in final reaction conditions.

7.4 50 mM $MgCl_2$ Solution (F-510MG)

Both Phusion Buffers supply 1.5 mM $MgCl_2$ at final reaction conditions. If higher $MgCl_2$ concentrations are desired, use 50 mM $MgCl_2$ solution to increase the $MgCl_2$ titer. Using the following equation, you can calculate the volume of 50 mM $MgCl_2$ needed to attain the final $MgCl_2$ concentration: [desired mM Mg]–[1.5 mM] = µL to add to a 50 µL reaction. For example, to increase the $MgCl_2$ concentration to 2.0 mM, add 0.5 µL of the 50 mM $MgCl_2$ solution. Because the PCR reactions can be quite sensitive to changes in the $MgCl_2$ concentration, it is recommended that the 50 mM $MgCl_2$ stock solution is diluted 1:5 (to 10 mM) to minimize pipetting errors.

7.5 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.

CERTIFICATE OF ANALYSIS

Endonuclease contamination assay

No endonuclease activity was observed after incubation of DNA polymerase with supercoiled plasmid DNA.

DNA amplification assay

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

Quality authorized by: Jurgita Zilinskiene

TECHNICAL SUPPORT:
EMEA: ts.molbio.eu@thermofisher.com
North America, Latin America & APAC: ts.molbio@thermofisher.com

NOTICE TO PURCHASE:

- The purchase price of this product includes a limited, non-transferable license under national patents from EP 0 547 920 B1, owned by New England Biolabs, Inc., to use this product. No other license under these patents is conveyed expressly or by implication to the purchaser by the purchase of this product.
- The purchase price of this product includes a limited, non-transferable license under U.S. and foreign patents owned by BIO-RAD Laboratories, Inc., to use this product. No other license under these patents is conveyed expressly or by implication to the purchaser by the purchase of this product.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

© 2012 Thermo Fisher Scientific, Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.