Phusion™ High–Fidelity DNA Polymerase

Catalog Numbers F530S and F530L

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Phusion™ High–Fidelity DNA Polymerase offers high performance for all major PCR applications. The Phusion™ High–Fidelity DNA Polymerase brings together a *Pyrococcus*–like enzyme with a processivity–enhancing domain. The Phusion™ High–Fidelity DNA Polymerase generates long amplicons with accuracy and speed, even on the most difficult templates. The high fidelity makes the Phusion™ High–Fidelity DNA Polymerase a superior choice for cloning. Using a *lacI*–based method modified from previous studies (see reference 1 on page 4), the error rate of Phusion™ High–Fidelity DNA Polymerase in Phusion™ HF Buffer is determined to be 4.4×10^{-7}, which is approximately 50 fold lower than that of the *Thermus aquaticus* DNA polymerase, and 6-fold lower than that of the *Pyrococcus furiosus* DNA polymerase.

The Phusion™ High–Fidelity 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 Phusion™ High–Fidelity DNA Polymerase is also capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb λ DNA.

Contents and storage

Component	F530S	F530L
Phusion™ DNA Polymerase, 2 U/µL	100 U 50 µL	500 U 250 µL
5X Phusion™ HF Buffer[1]	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

[1] Both 5X Phusion™ HF Buffer and 5X Phusion™ GC Buffer provide 1.5 mM $MgCl_2$ in final reaction concentration.

Important notes

- Use 98°C for denaturation (see “Initial denaturation” on page 3 and “Denaturation” on page 3).
- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read “Primer annealing” on page 3 carefully.
- Use 15–30 s/kb for extension. Do not exceed 1 min/kb (see “Extension” on page 4).
- Use Phusion™ High–Fidelity DNA Polymerase at 0.5–1.0 U per 50 µL reaction volume. Do not exceed 2 U/50 µL (see “Enzyme” on page 3).
- Use 200 µM of each dNTP. Do not use dUTP (see “Mg2+ and dNTP” on page 3).
- Phusion™ High–Fidelity DNA Polymerase produce blunt end DNA products.

Recommended protocol

- PCR reactions should be set up on ice.
- Prepare a master mix for the appropriate number of samples to be amplified.

- The Phusion™ High–Fidelity DNA Polymerase should be pipetted carefully and gently as the high glycerol content (50%) in the storage buffer may otherwise lead to pipetting errors.
- Due to the nature of the Phusion™ High–Fidelity 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, the Phusion™ High–Fidelity DNA Polymerase tends to work better at elevated denaturation and annealing temperatures.
- Follow the conditions listed in “Notes about cycling conditions” on page 3 when running your reactions.

1. Prepare PCR reactions. Add the following components in the order listed in the following table.

   Note:
   - It is critical that the Phusion™ High–Fidelity 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.
   - Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery.

Component	20 µL rxn	50 µL rxn	Final conc.
H_2O	add to 20 µL	add to 50 µL	
5X Phusion™ HF Buffer[1]	4 µL	10 µL	1X
10 mM dNTPs	0.4 µL	1 µL	200 µM each
Forward primer[2]	X µL	X µL	0.5 µM
Reverse primer [2]	X µL	X µL	0.5 µM
Template DNA	X µL	X µL	
(DMSO[3], optional)	(0.6 µL)	(1.5 µL)	(3%)
Phusion™ High–Fidelity DNA Polymerase	0.2 µL	0.5 µL	0.02 U/µL

[1] Optionally 5X GC Buffer can be used. See “Buffers” on page 3 for details.
[2] 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.
[3] 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.

2. Run the following program.

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	98°C	5–10 s	98°C	5–10 s	25–35
Annealing [1,2]	–	–	X°C	10–30 s	
Extension [3,4]	72°C	15–30 s/kb	72°C	15–30 s/kb	
Final extension	72°C	5–10 min	72°C	5–10 min	1
Hold	4°C	Hold	4°C	Hold	Hold

[1] See “Primer annealing” on page 3.
[2] For the 2–step protocol, there is no annealing step.
[3] See “Extension” on page 4.
[4] For the 2–step protocol, annealing and extansion are performed at the same temperature.

Notes about reaction components

Enzyme

The optimal amount of enzyme depends on the amount of template and the length of the PCR product. Usually 1 unit of Phusion™ High–Fidelity 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.

Buffers

Two buffers are provided with the enzyme: 5X Phusion™ HF Buffer and 5X Phusion™ GC Buffer. The error rate of Phusion™ High–Fidelity 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™ High–Fidelity 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 must be free of detergents, detergent–free reaction buffers are available for Phusion™ High–Fidelity DNA Polymerases (#F–520L, #F–521L).

Mg^{2+} and dNTP

The concentration of Mg^{2+} is critical since Phusion™ High–Fidelity 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™ High–Fidelity 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™ High–Fidelity DNA Polymerase there is no advantage of increasing dNTP concentrations. For optimal results always use 200 µM of each dNTP.

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.

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™ High–Fidelity 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 (see reference 2 on page 4).

Notes about cycling conditions

Initial denaturation

Denaturation should be performed at 98°C. Due to the high thermostability of Phusion™ High–Fidelity 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.

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.

Primer annealing

The optimal annealing temperature for Phusion™ High–Fidelity DNA Polymerase may differ significantly from that of *Taq*–based polymerases. Always use the Tm calculator and instructions on our website (**www.thermofisher.com/tmcalculator**) to determine the Tm values of primers and optimal annealing temperature.

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.

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.

Recommendations for cloning

When cloning fragments amplified with Phusion™ High–Fidelity 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 (#EP0402), for example. Incubate purified PCR product with 1x *Taq* buffer, 2.5 mM $MgCl_2$, 0.2 mM dATP and 1 U *Taq* DNA polymerase in 10 µL reaction mixture up to 30 min at 72°C. Before adding the overhangs it is very important to remove all the Phusion™ High–Fidelity DNA Polymerase by carefully purifying the PCR product, for example using Thermo Scientific™ GeneJET™ PCR Purification Kit (#K0701). Any remaining Phusion™ High–Fidelity DNA Polymerase will degrade the A overhangs, creating blunt ends again.

Component specifications

Phusion™ High–Fidelity DNA Polymerase (F-530)

Thermostable Phusion™ High–Fidelity DNA Polymerase is purified from an *E.coli* strain expressing the cloned Phusion™ High–Fidelity DNA Polymerase gene. Phusion™ High–Fidelity 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 dTTP, 200 µM each dATP, dGTP, dCTP, 0.4 MBq/ml [^{3}H] dTTP.

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.

5X GC buffer (F-519)

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

50 mM $MgCl_2$ solution (F-510MG)

Both 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.

Dimethyl sulfoxide DMSO, 100% (F-515)

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

References

1. Frey M. & Suppmann B. (1995) *Biochemica* 2: 34–35.
2. Chester N. & Marshak D.R. (1993) *Analytical Biochemistry* 209: 284–290.

Troubleshooting and FAQs

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
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Revision history: Pub. No. MAN0012393

Revision	Date	Description
E.0	9 July 2021	Corrected skus in contents and storage table from F553S and F553L to F530S and F530L.
D.0	28 January 2020	Moved troubleshooting content to thermofisher.com.
C.0	27 June 2018	Baseline for revision.