Phusion® High-Fidelity DNA Polymerase

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M0530L 005140316031

M0530L

500 units	**2,000 U/ml**	**Lot: 0051403**
RECOMBINANT	**Store at –20°C**	**Exp: 3/16**

Description: High Fidelity DNA Polymerases are important for applications in which the DNA sequence needs to be correct after amplification. Manufactured and quality controlled at New England Biolabs, Thermo Scientific® Phusion High-Fidelity DNA Polymerase offers both high fidelity and robust performance, and thus can be used for all PCR applications. Its unique structure, a novel *Pyrococcus*-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. Phusion DNA Polymerase is an ideal choice for cloning and can be used for long or difficult amplicons. With an error rate 50-fold lower than that of *Taq* DNA Polymerase and 6-fold lower than that of *Pyrococcus furiosus* DNA Polymerase (1), Phusion is one of the most accurate thermostable polymerases available. Phusion DNA Polymerase possesses 5´→ 3´ polymerase activity, 3´→ 5´ exonuclease activity and will generate blunt-ended products.

Phusion DNA Polymerase is supplied with standard 5X Phusion HF Buffer, as well as 5X Phusion GC Buffer, which can be used for complex or GC-rich templates. Each of these buffers contains $MgCl_2$ (1.5 mM at the final [1X] reaction concentration). Reactions can also be optimized using the provided DMSO or $MgCl_2$ solutions.

Source: An *E. coli* strain that carries the Phusion DNA Polymerase gene.

Applications:

- PCR
- Cloning
- Long or Difficult Amplification
- High-throughput PCR

Supplied in: 20 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 200 µg/ml BSA and 50% glycerol

Reagents Supplied with Enzyme:
5X Phusion HF Buffer
5X Phusion GC Buffer
100% DMSO
50 mM $MgCl_2$ Solution

Reaction Conditions: 1X Phusion HF or GC Buffer, DNA template, 0.5 µM primers, 200 µM dNTPs (not included), 3% DMSO (optional) and 1 unit of Phusion DNA Polymerase in a total reaction volume of 50 µl.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.

Unit Assay Conditions: 25 mM TAPS-HCl (pH 9.3 @ 25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM -mercaptoethanol, 200 µM dNTPs including [^{3}H]-dTTP and 400 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

7.5 kb Genomic DNA PCR: 30 cycles of PCR amplification in a 50 µl reaction containing 50 ng genomic DNA with 1.0 unit of Phusion DNA Polymerase in the presence of 200 µM dNTPs and 1.0 µM primers in Phusion HF Buffer results in the expected 7.5 kb product.

20 kb Lambda DNA PCR: 22 cycles of PCR amplification in a 50 µl reaction containing 10 ng Lambda DNA with 1.0 unit of Phusion DNA Polymerase in the presence of 200 µM dNTPs and 1.0 µM primers in Phusion HF Buffer results in the expected 20 kb product.

Endonuclease Activity: Incubation of a 50 µl reaction in NEBuffer 2 containing a minimum of 10 units of Phusion DNA Polymerase with 200 µM dNTPs and 1 µg of supercoiled ϕX174 DNA for 4 hours at either 37°C or 72°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

PCR

The following guidelines are provided to ensure successful PCR using Phusion DNA Polymerase. These guidelines cover routine PCR reactions. Amplification of templates with high GC content, high secondary structure, low template concentrations or long amplicons may require further optimization.

Reaction Setup: We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed and centrifuged prior to use. It is important to add Phusion DNA Polymerase last in order to prevent any primer degradation caused by the 3´→ 5´ exonuclease activity. Phusion DNA Polymerase may be diluted in 1X HF or GC Buffer just prior to use in order to reduce pipetting errors. **Please note that protocols with Phusion DNA Polymerase may differ from protocols with other standard polymerases. As such, conditions recommended below should be used for optimal performance.**

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Phusion HF or GC Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
DMSO (optional)	*(0.75 µl)*	*(1.5 µl)*	*(3%)*
Phusion DNA Polymerase	0.25 µl	0.5 µl	1.0 units/ 50 µl PCR
Template DNA	variable	variable	<250 ng
Nuclease-Free Water	to 25 µl	to 50 µl	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes from ice to a PCR machine with the block preheated to 98°C and begin thermocycling:

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
	45–72°C	10–30 seconds
	72°C	15–30 seconds/kb
Final Extension	72°C	5–10 minutes
Hold	4°C	

General Guidelines:

1. Template:
   Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	AMOUNT
Genomic	50 ng–250 ng
Plasmid or Viral	1 pg–10 ng

   If the template DNA is obtained from a cDNA synthesis reaction, the volume added should be less than 10% of the total reaction volume.

2. Primers:
   Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a PCR reaction using Phusion DNA Polymerase may be 0.2–1 µM, while 0.5 µM is recommended.

3. Mg^{++} and additives:
   Mg^{++} is critical to achieve optimal activity with Phusion DNA Polymerase. The final Mg^{++} concentration in 1X Phusion HF and GC Buffer is 1.5 mM. Excessive Mg^{++} can prevent full denaturation of DNA as well as cause non-specific binding of primers. The optimal Mg^{++} concentration is affected by dNTP concentration, the template being used and supplements that are added to the reaction. If chelators (e.g. EDTA) are present, it may be necessary to increase the Mg^{++} concentration. Mg^{++} can be optimized in 0.5 mM increments using the $MgCl_2$ provided.

   Amplification of difficult targets, such as those with GC-rich sequences or secondary structure, may be improved by the presence of additives such as DMSO (included). A final concentration of 3% DMSO is recommended, although concentration can be optimized in 2% increments. It is important to note that if a high concentration of DMSO is used, the annealing temperature must be lowered as it decreases the primer T_m (2). Phusion DNA polymerase is also compatible with other additives such as formamide or glycerol.

4. Deoxynucleotides:
   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide. Phusion cannot incorporate dUTP and is not recommended for use with uracil-containing primers or template.

5. Phusion DNA Polymerase Concentration:
   We generally recommend using Phusion DNA Polymerase at a concentration of 20 units/ml (1.0 units/50 µl reaction). However, the optimal concentration of Phusion DNA Polymerase may vary from 10–40 units/ml (0.5–2 units/50 µl reaction) depending on amplicon length and difficulty. Do not exceed 2 units/50 µl reaction, especially for amplicons longer than 5 kb.

(see other side)

CERTIFICATE OF ANALYSIS

6. Buffers
   5X Phusion HF Buffer and 5X Phusion GC Buffer are provided with the enzyme. HF buffer is recommended as the default buffer for high-fidelity amplification. For difficult templates, such as GC-rich templates or those with secondary structure, GC buffer can improve reaction performance. GC buffer should be used in experiments where HF buffer does not work. Detergent-free reaction buffers are also available for applications that do not tolerate detergents (e.g. microarray, DHPLC).

7. Denaturation:
   An initial denaturation of 30 seconds at 98°C is sufficient for most amplicons from pure DNA templates. Longer denaturation times can be used (up to 3 minutes) for templates that require it.

   During thermocycling, the denaturation step should be kept to a minimum. Typically, a 5–10 second denaturation at 98°C is recommended for most templates.

8. Annealing:
   Annealing temperatures required for use with Phusion tend to be higher than with other PCR polymerases. **The NEB T_m calculator (www.neb.com/TmCalculator) should be used to determine the annealing temperature when using Phusion.** Typically, primers greater than 20 nucleotides in length anneal for 10–30 seconds at 3°C above the T_m of the lower T_m primer. If the primer length is less than 20 nucleotides, an annealing temperature equivalent to the T_m of the lower primer should be used. A temperature gradient can also be used to optimize the annealing temperature for each primer pair. For two-step cycling, the gradient can be set as high as the extension temperature.

   For high T_m primer pairs, two-step cycling without a separate annealing step can be used.

9. Extension:
   The recommended extension temperature is 72°C. Extension times are dependent on amplicon length and complexity. Generally, an extension time of 15 seconds per kb can be used. For complex amplicons, such as genomic DNA, an extension time of 30 seconds per kb is recommended. Extension time can be increased to 40 seconds per kb for cDNA templates, if necessary.

10. Cycle number:
    Generally, 25–35 cycles yields sufficient product.

11. 2-step PCR:
    When primers with annealing temperatures ≥ 72°C are used, a 2-step thermocycling protocol is recommended.

    Thermocycling Conditions for a Routine 2-Step PCR:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
	72°C	15–30 seconds/kb
Final Extension	72°C	5–10 minutes
Hold	4°C	

12. PCR product:
    The PCR products generated using Phusion DNA Polymerase have blunt ends; if cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, then DNA should be purified prior to A-addition, as Phusion DNA Polymerase will degrade any overhangs generated.

    Addition of an untemplated -dA can be done with *Taq* DNA Polymerase (NEB #M0267) or Klenow exo$^-$ (NEB #M0212).

References:

1. Frey, B. and Suppman, B. (1995) *BioChemica*, 2, 34–35.
2. Chester, N. and Marshak, D.R. (1993) *Analytical Biochemistry*, 209, 284–290.

Companion Products Sold Separately:

Phusion HF Buffer Pack
#B0518S 6.0 ml

Phusion GC Buffer Pack
#B0519S 6.0 ml

Detergent-free Phusion HF Buffer Pack
#B0520S 6.0 ml

Detergent-free Phusion GC Buffer Pack
#B0521S 6.0 ml

Phusion High-Fidelity PCR Kit
#E0553S 50 reactions
#E0553L 200 reactions

Phusion High-Fidelity PCR Master Mix with HF Buffer
#M0531S 100 reactions
#M0531L 500 reactions

Phusion High-Fidelity PCR Master Mix with GC Buffer
#M0532S 100 reactions
#M0532L 500 reactions

Deoxynucleotide Solution Set
#N0446S 25 µmol of each

Deoxynucleotide Solution Mix
#N0447S 8 µmol of each
#N0447L 40 µmol of each

Magnesium Chloride ($MgCl_2$) Solution
#B9021S 6.0 ml

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