

**Phusion®**  
Hot Start Flex  
DNA Polymerase



**M0535S**



**100 units      2,000 U/ml      Lot: 0021411**  
**RECOMBINANT      Store at -20°C      Exp: 11/16**

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DNA Polymerase.

**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 Hot Start Flex DNA Polymerase offers robust, high fidelity performance and room temperature reaction setup. The addition of an aptamer-based inhibitor allows room temperature reaction setup. Phusion Hot Start Flex 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<sub>2</sub> (1.5 mM at the final [1X] reaction concentration). Reactions can also be optimized using the provided DMSO or MgCl<sub>2</sub> solutions.

**Note:** Phusion Hot Start Flex DNA Polymerase is unlike other enzymes and care must be taken when designing cycling protocols. To determine the optimal annealing temperatures for a given set of primers, use of the **NEB T<sub>m</sub> Calculator** is highly recommended ([www.neb.com/TmCalculator](http://www.neb.com/TmCalculator)).

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

**Applications:**

- High-specificity 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<sub>2</sub> 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 Hot Start Flex 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<sub>2</sub>, 1 mM β-mercaptoethanol, 200 µM dNTPs including [<sup>3</sup>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 Hot Start Flex 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 Hot Start Flex 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.

**Hot Start-Specific Genomic DNA PCR:** 25 cycles of PCR amplification in a 25 µl reaction containing 50 ng genomic DNA with 0.5 units of Phusion Hot Start Flex DNA Polymerase in the presence of 200 µM dNTPs and 0.5 µM primers in 1X Phusion GC Buffer results in the expected 665 bp product, free of non-specific amplification products after pre-incubation at room temperature for 1 hour.

**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 Hot Start Flex 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:** *Phusion Hot Start Flex DNA Polymerase is inhibited at room temperature, allowing flexible reaction set up (RT or ice).* All components should be mixed and spun down prior to use. Phusion Hot Start Flex 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 Hot Start Flex 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
Nuclease-Free water	to 25 µl	to 50 µl	
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
Template DNA	variable	variable	< 250 ng
DMSO (optional)	(0.75 µl)	(1.5 µl)	3%
Phusion Hot Start Flex DNA Polymerase	0.25 µl	0.5 µl	1.0 unit/50 µl PCR

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 to a PCR machine and begin thermocycling. *Phusion Hot Start Flex DNA Polymerase does not require a separate activation step. Standard Phusion cycling conditions are recommended.*

**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	

\*To determine the optimal annealing temperature for a given set of primers, use of the NEB T<sub>m</sub> Calculator is highly recommended ([www.neb.com/TmCalculator](http://www.neb.com/TmCalculator)).

**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.

(see other side)

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 experiment using Phusion Hot Start Flex DNA Polymerase may be 0.2–1  $\mu\text{M}$ , while 0.5  $\mu\text{M}$  is strongly recommended.

3.  $\text{Mg}^{++}$  and additives:  $\text{Mg}^{++}$  is critical to achieve optimal activity with Phusion Hot Start Flex DNA Polymerase. The final  $\text{Mg}^{++}$  concentration in 1X Phusion HF and GC Buffer is 1.5 mM. Excessive  $\text{Mg}^{++}$  can prevent full denaturation of DNA as well as cause non-specific binding of primers. The optimal  $\text{Mg}^{++}$  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  $\text{Mg}^{++}$  concentration.  $\text{Mg}^{++}$  can be optimized in 0.5 mM increments using the  $\text{MgCl}_2$  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 DMSO decreases the primer  $T_m$  (2). Phusion Hot Start Flex DNA Polymerase is also compatible with other additives such as formamide or glycerol.

4. Deoxynucleotides: The final concentration of dNTPs is typically 200  $\mu\text{M}$  of each deoxynucleotide. Phusion Hot Start Flex cannot incorporate dUTP and is not recommended for use with uracil-containing primers or template.

5. Phusion Hot Start Flex DNA Polymerase Concentration: We generally recommend using Phusion Hot Start Flex DNA Polymerase at a concentration of 20 units/ml (1.0 unit/50  $\mu\text{l}$  reaction). However, the optimal concentration of Phusion Hot Start Flex DNA Polymerase may vary from

10–40 units/ml (0.5–2 units/50  $\mu\text{l}$  reaction) depending on amplicon length and difficulty. Do not exceed 2 units/50  $\mu\text{l}$  reaction, especially for amplicons longer than 5 kb.

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. Detergent-free reaction buffers are also available for applications that do not tolerate detergents (e.g. microarray, DHPLC).

7. Denaturation: *Phusion Hot Start Flex DNA Polymerase does not require a separate activation step.* 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: Optimal annealing temperatures for Phusion Hot Start Flex DNA Polymerase tend to be higher than for other PCR polymerases. **The NEB  $T_m$  calculator ([www.neb.com/TmCalculator](http://www.neb.com/TmCalculator)) should be used to determine the annealing temperature when using Phusion products.** Typically, for primers > 20 nt, use a 10–30s annealing step at 3°C above the  $T_m$  of the lower  $T_m$  primer. For primers < 20 nt, 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 yield sufficient product.

11. 2-step PCR: When primers with annealing temperatures  $\geq 72^\circ\text{C}$  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 Hot Start Flex DNA Polymerase have blunt ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, the DNA should be purified prior to A-addition, as Phusion Hot Start Flex DNA Polymerase will degrade any overhangs generated.

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

#### References:

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

#### Companion Products Sold Separately:

Phusion Hot Start Flex 2X Master Mix #M0536S	100 reactions
#M0536L	500 reactions
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

Magnesium Chloride ( $\text{MgCl}_2$ ) Solution #B9021S 6.0 ml

Phusion High-Fidelity DNA Polymerase #M0530S 100 units  
#M0530L 500 units

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

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

Deoxynucleotide Solution Set #N0446S 25  $\mu\text{mol}$  of each

Deoxynucleotide Solution Mix #N0447S 8  $\mu\text{mol}$  of each  
#N0447L 40  $\mu\text{mol}$  of each



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