Phusion™ Plus DNA Polymerase

Catalog Numbers F630S, F630L, F630XL

Pub. No. MAN0025053 Rev. A.0

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™ Plus DNA Polymerase is a proofreading DNA polymerase that combines a novel Pyrococcus-like enzyme with a processivity-enhancing domain and universal primer annealing feature. The polymerase is inactive at ambient temperatures due to an Affibody™ molecule mediated hot start mechanism, allowing reaction setup and storage of pre-assembled PCR reactions at room temperature. Enzyme activity is restored after the initial denaturation step.

Primer annealing is performed at 60°C because proprietary additives in the reaction buffer stabilize primer-template duplexes during annealing, and eliminate the need to optimize annealing temperature for each primer pair.

The polymerase is ideal for applications where accuracy is important (cloning, sequencing, site directed mutagenesis), and possesses the following characteristics:

- 5´→3´ DNA polymerase activity.
- 3´→5´ exonuclease activity.
- Generates blunt end amplification products.
- Amplifies up to 10 kb from genomic DNA, and 20 kb from low complexity DNA.
- Works with both AT and GC rich targets (Phusion™ GC Enhancer is provided for amplicons with >65% GC content).
- >100X fidelity compared to *Taq* polymerase.

Contents and storage

Component	F630S 100 reactions	F630L 500 reactions	F630XL 4 × 500 reactions	Storage
Phusion™ Plus DNA Polymerase	50 µL	250 µL	4 × 250 µL	–25°C to –15°C
Phusion™ Plus Buffer	1.25 mL	5 × 1.25 mL	20 × 1.25 mL	
Phusion™ GC Enhancer	1.25 mL	4 × 1.25 mL	16 × 1.25 mL	

General guidelines

- Use 98°C for denaturation.
- Use 15–30 s/kb for extension.
- Use 200 µM of each dNTP. **Do not use dUTP** (The polymerase cannot read through dUTP-derivatives or dITP in the template strand, thus primers and dNTP mixes containing such nucleotides are not compatible).
- Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. Prepare a master mix for the appropriate number of samples to be amplified.
- Pipette polymerase carefully and gently, as the high glycerol content (50%) in the storage buffer may otherwise lead to pipetting errors.
- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.

Required materials not supplied

- Template: genomic DNA, plasmid, phage DNA, cDNA
- Forward and reverse primers
- 10 mM dNTP Mix (Cat. No. R0191)
- TopVision Agarose Tablets (Cat no. R2801)
- GeneRuler 1 kb DNA Ladder (Cat.no. SM0311)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- Water, nuclease-free

Perform PCR

1. Prepare reaction by adding the following components in the order listed in the following table.

Component	20 µL rxn	50 µL rxn	Final conc.
5X Phusion™ Plus Buffer [1]	4 µL	10 µL	1X
Forward primer	*x* µL	*x* µL	0.5 µM [2]
Reverse primer	*x* µL	*x* µL	0.5 µM [2]
10 mM dNTPs	0.4 µL	1 µL	200 µM each
Template DNA	*x* µL	*x* µL	0.01–10 ng plasmid 5–100 ng genomic DNA
5X Phusion™ GC Enhancer [3]	4 µL	10 µL	1X
Phusion™ Plus DNA Polymerase	0.2 µL	0.5 µL	—
Water, nuclease free	add to 20 µL	add to 50 µL	—

[1] Provides 1.7 mM $MgCl_2$ at 1X concentration.
[2] Reduce the primer concentration to 0.2 µM final concentration when amplifying >5 kb targets from genomic DNA and for multiplex reactions.
[3] (Optional) recommended only for targets with >65% GC content.

2. Run a thermal cycler program set to the following parameters according to the protocol to be performed.

   a. 3-step protocol

Cycle step	Temp.	Time	Cycles
Initial Denaturation	98°C	30 s	1
Denaturation Annealing Extension	98°C 60°C 72°C	5–10 s 10 s 15–30 s/kb	25–35
Final extension	72°C 4°C	5 min Hold	1 Hold

   b. 2-step protocol (for primers >30 nt in length)

Cycle step	Temp.	Time	Cycles
Initial Denaturation	98°C	30 s	1
Denaturation Annealing/extension	98°C 72°C	5–10 s 15–30 s/kb	25–35
Final extension	72°C 4°C	5 min Hold	1 Hold

Optimization strategies

Primers

- Design 18- to 35-mers with 40–60% GC content. Avoid primer pairs with complementarity at 3' ends or >10°C melting temperature (T_m) difference.
- Verify primer complementarity to a single template region using programs for sequence alignment. Online primer design programs such as the Invitrogen™ OligoPerfect™ Designer can be helpful.
- Recommended final primer concentration is 0.5 µM and can be varied in a range of 0.1–1.0 µM, if needed. Lower primer concentrations (0.2 µM final) are recommended for amplification of >5 kb targets from high complexity DNA and multiplex reactions.

Template

- **Low complexity DNA:** Optimal amount of low complexity DNA (plasmid, phage or BAC DNA) is 0.01–10 ng per 50 µL reaction, although it can be varied from 0.1 pg to 50 ng per 50 µL reaction.
- **Genomic DNA:** Optimal amount of genomic DNA is 5–100 ng per 50 µL reaction, although it can be varied from 0.1–250 ng per 50 µL reaction. Higher template amount is recommended for long targets.
- **cDNA:** Optimal amount of cDNA is 0.1–1 µL of the first-strand reaction mixture per 50 µL reaction.

$MgCl_2$

Phusion™ Plus reaction buffer provides 1.7 mM $MgCl_2$ in the final reaction. If the primers and/or the template contain chelators such as EDTA or EGTA, the apparent Mg^{2+} optimum may be shifted to higher concentrations. $MgCl_2$ concentration can be increased up to 4.5 mM final concentration in such cases.

Cycling parameters

The total number of PCR cycles can vary from 15 to 40, depending on target length and template amount. For low complexity templates 20–25 PCR cycles is typical; 30–35 cycles are recommended for genomic DNA.

Denaturation

- Use 98°C for denaturation. Make sure that heated lid temperature is set several degrees above 98°C to avoid sample condensation.
- 30-second initial denaturation at 98°C is sufficient for most templates. The initial denaturation time can be increased up to 5 minutes if necessary.

Annealing

- Due to unique isostabilizing molecules in the reaction buffer, 60°C annealing temperature works for most primers.
- The 2-step protocol is recommended when primers without non-complementary parts are >30 nt in length, e.g., primers for site-specific mutagenesis. In the 2-step protocol the combined annealing/extension step should be performed at 72°C.
- If amplification does not give satisfactory results, we recommend a temperature gradient. The annealing temperature can be optimized using Applied Biosystems™ thermal cyclers, such as the ProFlex™ PCR System or the VeritiPro™ Thermal Cycler featuring VeriFlex™ technology.

Extension

- Extension time depends on amplicon length and complexity. For low complexity DNA (e.g., plasmid, phage 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.
- The extension step can be prolonged up to 90 sec/kb for targets up to 5 kb without negative effect on specificity. This allows to amplify shorter and longer amplicons together using the same protocol.

Troubleshooting

Observation	Possible cause	Recommended action
No product or low yield	Sub-optimal cycling parameters.	Run a temperature gradient to determine optimal annealing temperature.
		Increase the total number of cycles.
	Problem with reaction mix.	Repeat the PCR and make sure that there are no pipetting errors.
		Use fresh, high-quality dNTPs. Do not use dNTP mix containing dUTP.
		Check primer design and concentration. Do not use primers containing dUTP or dITP.
		Titrate template amount, both not enough and too much template can compromise PCR results.
Non-specific products or smeared bands	Sub-optimal cycling parameters.	Run a temperature gradient to determine optimal annealing temperature.
		Decrease extension time.
		Reduce the total number of cycles.
	Primer concentration too high.	Reduce primer concentration.

Documentation and support

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

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

Limited product warranty

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Revision	Date	Description
A.0	6 April 2021	Baseline for revision.