Phusion High-Fidelity DNA Polymerases

Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerases offer very high fidelity, speed, and yield for all PCR applications.

General instructions

- Due to the unique nature of Phusion DNA polymerases, always use the T_m calculator on our website to determine optimal annealing temperature (**thermofisher.com/tmcalculator**).
- Use 98°C for denaturation.
- Use 15–30 sec/kb for extension. Do not exceed 1 min/kb.
- Use Phusion DNA polymerases at 0.5–1.0 U per 50 µL reaction volume. Do not exceed 2 U per 50 µL reaction volume.
- Use 200 µM of each dNTP.
- If uracil is present in the dNTP mix or DNA template, use Thermo Scientific™ Phusion™ U Hot Start DNA Polymerase.

Note: Phusion DNA polymerases produce blunt-end DNA products.

Choosing the right Phusion product

		Phusion High-Fidelity DNA Polymerase (Cat. No. F530S)	Phusion Hot Start II High-Fidelity DNA Polymerase (Cat. No. F549S)	Phusion Flash High-Fidelity DNA Polymerase (Cat. No. F548S)	Phusion U Hot Start DNA Polymerase (Cat. No. F555S)	Phusion U Multiplex PCR Master Mix (Cat. No. F562S)
Characteristics	Blunt or 3′-A end	Blunt	Blunt	Blunt	Blunt	Blunt
	Target length, genomic/phage DNA	≤16/20 kb	≤16/20 kb	≤16/20 kb	≤7.5/20 kb	≤2.5/2.5 kb
	Hot start	No	Yes	Yes	Yes	Yes
	Recommended extension time	15–30 sec/kb	15–30 sec/kb	15 sec/kb	15–30 sec/kb	15–30 sec/kb
	Fidelity vs. *Taq*	52x	52x	25x	25x	NA
	dUTP tolerance	No	No	No	Yes	Yes
Formats	Enzyme*	✓	✓		✓	
	Green buffer**	✓	✓			
	Master mix†	✓	✓	✓	✓	✓
	Complete kit‡	✓				

* DNA polymerase, buffer, DMSO, and $MgCl_2$.
** DNA polymerase supplied with Phusion Green Buffer, which includes a density reagent and two tracking dyes for direct loading on gel.
† 2X master mix format.
‡ All the necessary PCR components, including control template and primers.

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Reaction setup

Component	50 µL reaction	20 µL reaction	Final concentration
5X Phusion buffer*	10 µL	4 µL	1X
10 mM dNTPs*	1 µL	0.4 µL	200 µM each
Primer A	x µL	x µL	0.5 µM
Primer B	y µL	y µL	0.5 µM
Template DNA	z µL	z µL	–
DMSO (optional)	(1.5 µL)	(0.6 µL)	(3%)
Phusion DNA polymerase	0.5 µL	0.2 µL	0.02 U/µL
Water	To 50 µL total	To 20 µL total	–

* If you are using any of the Phusion PCR master mix products, add 25 or 10 µL of the 2X master mix (depending on the final reaction volume). Do not add dNTPs.

Cycling instructions for Phusion and Phusion Hot Start II High-Fidelity DNA Polymerases

	2-step protocol		3-step protocol		
Cycle step	Temperature	Time	Temperature	Time	Cycles
Initial denaturation	98°C	30 sec	98°C	30 sec	1
Denaturation	98°C	5–10 sec	98°C	5–10 sec	25–35
Annealing*	–	–	X°C*	10–30 sec	
Extension	72°C	15–30 sec/kb	72°C	15–30 sec/kb	
Final extension	72°C	5–10 min	72°C	5–10 min	1
	4°C	Hold	4°C	Hold	

* Depends on the primer T_m values. Use the T_m calculator at **thermofisher.com/tmcalculator**

Cycling instructions for Phusion Flash High-Fidelity PCR Master Mix

	2-step protocol		3-step protocol		
Cycle step	Temperature	Time	Temperature	Time	Cycles
Initial denaturation	98°C	10 sec	98°C	10 sec	1
Denaturation*	98°C	0 or 1 sec	98°C	0 or 1 sec	30
Annealing**	–	–	50–72°C	5 sec	
Extension	72°C	15 sec/kb	72°C	15 sec/kb	
Final extension	72°C	1 min	72°C	1 min	1
	4°C	Hold	4°C	Hold	

* A very short denaturation step is recommended. If the PCR instrument used does not accept 0 sec as a value, then a 1 sec value can be programmed.
** Depends on the primer T_m values. Use the T_m calculator at **thermofisher.com/tmcalculator**

Find out more at **thermofisher.com/phusion**

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