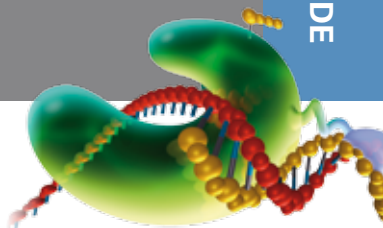


Thermo Scientific Phusion High-Fidelity DNA Polymerases



Phusion® High-Fidelity DNA Polymerases offer extreme fidelity, speed and yield for all PCR applications. Due to the unique nature of Phusion DNA Polymerases, please pay special attention to the guidelines listed below.

General instructions

- Use 98°C for denaturation.
- Use 15-30 s/kb for extension. Do not exceed 1 min/kb.
- Use Phusion High-Fidelity DNA Polymerases at 0.5-1.0 U per 50 µl reaction volume. Do not exceed 2 U/50 µl.
- Use 200 µM of each dNTP. Do not use dUTP.
- Note: The annealing rules are different from many common DNA polymerases.
- Note: Phusion DNA Polymerases produce blunt end DNA products.

Ordering information

F-530S/L	Phusion High-Fidelity DNA Polymerase
F-549S/L	Phusion Hot Start II High-Fidelity DNA Polymerase
F-548S/L	Phusion Flash High-Fidelity PCR Master Mix
F-531S/L	Phusion High-Fidelity PCR Master Mix with HF Buffer
F-532S/L	Phusion High-Fidelity PCR Master Mix with GC Buffer
F-553S/L	Phusion High-Fidelity PCR Kit

www.thermoscientific.com/phusion

Pipetting instructions (in order)

Component	50 μ l reaction	20 μ l reaction	Final conc.
H ₂ O	add to 50 μ l	add to 20 μ l	-
5x Phusion HF Buffer*	10 μ l	4 μ l	1x
10 mM dNTPs*	1 μ l	0.4 μ l	200 μ M each
primer A	x μ l	x μ l	0.5 μ M
primer B	x μ l	x μ l	0.5 μ M
template DNA	x μ l	x μ l	-
(DMSO, optional)	(1.5 μ l)	(0.6 μ l)	(3 %)
Phusion DNA Polymerase	0.5 μ l	0.2 μ l	0.02 U/ μ l

* If you are using any of the Phusion PCR Master Mix products, add 25 or 10 μ l of the 2x Master Mix (depending on the final reaction volume). Do not add dNTPs.

Cycling instructions

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*	-	-	X°C	10-30 s	
Extension	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
	4°C	hold	4°C	hold	

* Depends on primer T_m's. Use the T_m calculator at www.thermoscientific.com/pcrwebtools.

Cycling instructions for Phusion Flash PCR Master Mix

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	10 s	98°C	10 s	1
Denaturation	98°C	0 or 1 s	98°C	0 or 1 s	30
Annealing	-	-	50-72°C	5 s	
Extension	72°C	15 s/kb	72°C	15 s/kb	
Final extension	72°C	1 min	72°C	1 min	1
	4°C	hold	4°C	hold	

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