Thermo Scientific Phusion
High-Fidelity DNA Polymerases

Thermo Scientific™ 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

- Note: The annealing rules are different from many common DNA polymerases
  (for more details please visit www.thermoscientific.com/pcrwebtools).
- 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: Phusion DNA Polymerases produce blunt end DNA products.

Ordering information

Product	Cat. No.	Quantity	Cat. No.	Quantity
Phusion High-Fidelity DNA Polymerase	F-530S	100 U (2 U/µL)	F-530L	500 U (2 U/µL)
Phusion Hot Start II High-Fidelity DNA Polymerase	F-549S	100 U (2 U/µL)	F-549L	500 U (2 U/µL)
Phusion Green High-Fidelity DNA Polymerase	F-534S	100 U (2 U/µL)	F-534L	500 U (2 U/µL)
Phusion Green Hot Start II High-Fidelity DNA Polymerase	F-537S	100 U (2 U/µL)	F-537L	500 U (2 U/µL)
Phusion Flash High-Fidelity PCR Master Mix	F-548S	100 reactions in 20 µL volume	F-548L	500 reactions in 20 µL volume
Phusion High-Fidelity PCR Master Mix with HF Buffer	F-531S	100 reactions in 50 µL volume	F-531L	500 reactions in 50 µL volume
Phusion High-Fidelity PCR Master Mix with GC Buffer	F-532S	100 reactions in 50 µL volume	F-532L	500 reactions in 50 µL volume
Phusion High-Fidelity PCR Kit	F-553S	50 reactions in 50 µL volume	F-553L	200 reactions in 50 µL volume

Thermo Scientific Phusion
High-Fidelity DNA Polymerases

Pipetting instructions (in order)

Component	50 µL reaction	20 µL reaction	Final concentration
Water	add to 50 µL	add to 20 µL	–
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	x µL	x µL	0.5 µM
Template DNA	x µL	x µL	–
(DMSO, optional)	(1.5 µL)	(0.6 µL)	(3 %)
Phusion DNA Polymerase	0.5 µL	0.2 µL	0.02 U/µL

* 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

Cycle step	2-step protocol		3-step protocol		Cycles
	Temperature	Time	Temperature	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 the primer Tm values. Use the Tm calculator at www.thermoscientific.com/pcrwebtools.

Cycling instructions for Phusion Flash PCR Master Mix

Cycle step	2-step protocol		3-step protocol		Cycles
	Temperature	Time	Temperature	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	

For more information or to place an order:
www.thermoscientific.com/phusion

© 2013 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc., and its subsidiaries.

LabAid

Europe Customer Service
cs.molbio.eu@thermofisher.com

North America Customer Service
cs.molbio@thermofisher.com

Literature #MB1330

Thermo SCIENTIFIC