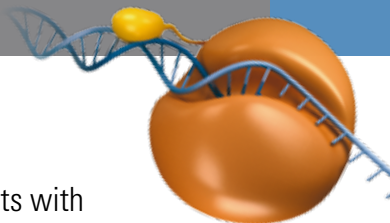


# Thermo Scientific Phire Hot Start II DNA Polymerase



Phire<sup>®</sup> Hot Start II DNA Polymerase provides high yields of specific products with time-saving PCR protocols. To achieve the best results, please pay attention to the guidelines listed below.

## General instructions

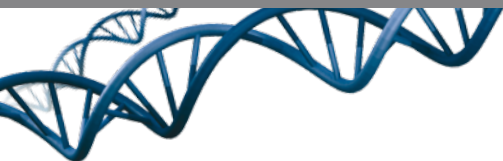
- Use 98°C for denaturation.
- Use 0.4 µl of enzyme per 20 µl reaction and 1 µl per 50 µl reaction.
- Use 200 µM of each dNTP. Do not use dUTP.
- Note: The annealing rules are different from many common DNA polymerases.
- Note: Phire Hot Start II DNA Polymerase produces blunt end DNA products.

## Ordering information

### Phire Hot Start II DNA Polymerase

F-122S	200 reactions à 50 µl (200 µl)
F-122L	1000 reactions à 50 µl (1.0 ml)

[www.thermoscientific.com/phire](http://www.thermoscientific.com/phire)



## Pipetting instructions (in order)

Component	50 $\mu$ l reaction	20 $\mu$ l reaction	Final conc.
H <sub>2</sub> O	add to 50 $\mu$ l	add to 20 $\mu$ l	-
5x Phire <sup>®</sup> Reaction Buffer	10 $\mu$ l	4 $\mu$ l	1x
10 mM dNTPs	1 $\mu$ l	0.4 $\mu$ l	200 $\mu$ M each
Primer A	x $\mu$ l	x $\mu$ l	0.5 $\mu$ M
Primer B	x $\mu$ l	x $\mu$ l	0.5 $\mu$ M
Template DNA*	x $\mu$ l	x $\mu$ l	-
Phire <sup>®</sup> Hot Start II DNA Polymerase	1 $\mu$ l	0.4 $\mu$ l	-

\* Low complexity DNA 1 pg-10 ng per 20  $\mu$ l, 2.5 pg-25 ng per 50  $\mu$ l. High complexity genomic DNA 10-100 ng or 25-250 ng depending on the volume.

## 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 s	98°C	5 s	25-35
Annealing*	-	-	X°C	5 s	
Extension	72°C	10-15 s/kb	72°C	10-15 s/kb	
Final extension	72°C	1 min	72°C	1 min	1
	4°C	hold	4°C	hold	

\* Depends on the primer T<sub>m</sub> values. Use the T<sub>m</sub> calculator at [www.thermoscientific.com/pcrwebtools](http://www.thermoscientific.com/pcrwebtools).

### Technical Support :

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