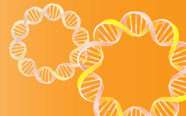
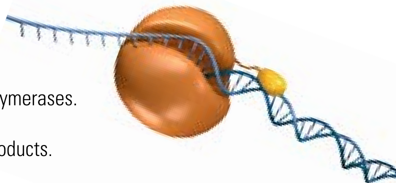
Thermo Scientific Phire
Hot Start II DNA Polymerases



Thermo Scientific™ Phire™ Hot Start II DNA Polymerase outperforms every *Taq*-based hot start polymerase on the market. This polymerase is significantly faster, extremely robust, and also capable of amplifying long DNA fragments with high yields. These features are achieved through advanced protein engineering of the polymerase. To achieve the best results, please pay attention to the guidelines listed below.
The new Phire Green format is a combination of Phire Hot Start II DNA Polymerase and 5X Green Reaction Buffer. The buffer includes a density reagent and two tracking dyes for direct loading of PCR products on gels.

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. (for more details please visit www.thermoscientific.com/pcrwebtools).
- Note: Phire Hot Start II DNA Polymerase produces blunt end DNA products.



Ordering information

Product	Cat. No.	Quantity
Phire Hot Start II DNA Polymerase	F-122S	200 reactions of 50 µL (200 µL)
	F-122L	1000 reactions of 50 µL (1.0 mL)
Phire Green Hot Start II DNA Polymerase	F-124S	200 reactions of 50 µL (200 µL)
	F-124L	1000 reactions of 50 µL (1.0 mL)

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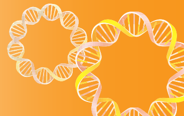

Pipetting instructions (in order)

Component	50 µL reaction	20 µL reaction	Final concentration
Water	add to 50 µL	add to 20 µL	–
5X Phire Reaction Buffer or 5X Phire Green Reaction 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	–
Phire Hot Start II DNA Polymerase	1 µL	0.4 µL	–

* High complexity genomic DNA: 10-100 ng per 20 µL reaction volume, or 25-250 ng per 50 µL reaction volume.
Low complexity DNA: 1 pg-10 ng per 20 µL reaction volume, or 2.5 pg-25 ng per 50 µL reaction volume.

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 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 Tm values. Use the Tm calculator at www.thermoscientific.com/pcrwebtools.

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

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LabAid

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

North America Customer Service
cs.molbio@thermofisher.com

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