

Phire[®]

Hot Start II DNA Polymerase

Finnzymes' Phire[®] 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)



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 Phire [®] Reaction 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	
Phire [®] Hot Start II DNA Polymerase	1 μ l	0.4 μ l	

* Low complexity DNA 1 pg-10 ng per 20 μ l, 2.5 pg-25 ng per 50 μ 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_m values. Use the T_m calculator on Finnzymes' website (www.finnzymes.com).

www.finnzymes.com

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