Phoenix Hot Start
Taq DNA Polymerase

Kit Contents

Part Number	P7590L	P7590S
Concentration	5,000 U/mL	5,000 U/mL
Unit Size	500 U	100 U
5X Phoenix Hot Start Taq Buffer	4X 1.5 mL	1X 1.5 mL
5X Phoenix Hot Start GC Buffer	2X 1.5 mL	1X 1.5 mL

Polymerase Properties

Molecular Weight: 94 kDa
Optimum Extension Temperature: 66 - 72°C
Extension Rate: 60 seconds per kilobase at 72˚C
Proofreading (3'-5' exo): No
Nick-translation (5'-3' exo): Yes
Strand Displacement: No
Thermostability: Moderate thermostability
Extends from a nick: Yes

Common Applications

Routine PCR amplification up to 5 kb, high-throughput PCR, primer extension, multiplex PCR, RT-PCR, and Taqman® and SYBR® Green based real time qPCR.

TaqMan is a registered trademark of Roche Molecular Systems, Inc.
SYBR Green is a registered trademark of Molecular Probes, Inc.

Protocol

General precautions should be taken when setting up a PCR, including steps to avoid cross-contamination, gentle pipetting, and thorough mixing and brief centrifugation after all components are added to the reaction. The following procedure can be used as a guideline. Reactions may need to be optimized individually depending on the desired result.

Reaction setup (for 50 µL)*

Component	Volume (µL)	Final Concentration
Sterile H_2O	x	
5X Phoenix Hot Start Taq buffer or GC buffer[1]	10	1X
10 mM dNTP mix	1	200 µM each
Primer 1[2]	x	0.2 µM
Primer 2[2]	x	0.2 µM
DNA template[3]	x	See usage note #3
Phoenix Hot Start Taq[4]	0.2	0.02 U/µL (or 1U)

* Volumes can be adjusted as needed.

Usage Notes:

1. 5X Phoenix Hot Start Taq buffer should be used as the default buffer. For GC-rich and difficult templates, use 5X Phoenix Hot Start GC buffer.

2. A final concentration of 0.2 µM is recommended for each primer, but can be varied in the range of 0.2 - 1 µM.

3. Recommended template quantities:

Complexity	Source Example	Guideline
Low	Plasmid, Virus, BAC	1 pg – 10 ng
High	Genomic DNA	10 - 100 ng

4. One unit of enzyme is usually sufficient for amplifying most targets, but more may be required (up to 2.5 units) in multiplex PCR or to increase yields of difficult or long targets.

5. Both 5X Phoenix Hot Start Taq buffer and GC buffer are formulated so that they will provide 2 mM Mg^{2+} in the final reaction (i.e. when diluted to 1X). In cases where additional Mg^{2+} optimization is required, adjust the final Mg^{2+} concentration in 0.2 mM steps.

Typical cycling conditions**

Step	Temperature	Time	Cycles
Initial Denaturation***	94°C	30 sec -3 min	1
Denaturation	94°C	30 sec	
Annealing	Varies	30 sec	25 - 40
Extension	72°C	60 sec/kb	
Final Extension	72°C	5 min	1
	4°C	hold	

** Cycling conditions may need to be optimized, depending on the amplicon of interest.

*** Required for template denaturation and activation of Phoenix Hot Start Taq Polymerase.

Frequently Asked Questions and Troubleshooting

For Frequently Asked Questions (FAQ) and troubleshooting please visit www.enzymatics.com