Product Information	
Phoenix Hot Start Taq DNA Polymerase	
Part Number	P7590L
Concentration	5000 U/mL
Unit Size	500 U
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

Product Description: Phoenix Hot Start Taq DNA Polymerase is a recombinant, thermostabile Taq DNA polymerase complexed with a thermolabile, neutralizing antibody that blocks the 5'→ 3' polymerase activity prior to the initial DNA denaturation step of PCR (1,2). Such antibody-mediated Hot-Start capability enhances the overall specificity, sensitivity and yield of the PCR by reducing nonspecific amplification and primer-dimer formation prior to PCR cycling, and allows the convenience of reaction set up at room temperature. When the temperature of the PCR reaction mix reaches ≥94°C during the initial DNA denaturing step of PCR cycling, activity of the Taq DNA polymerase is fully restored. Phoenix Hot Start Taq DNA Polymerase, like standard Taq DNA polymerase, also has 5'→3' exonuclease activity, but lacks any detectable 3'→5' exonuclease activity.

Product Specifications						
P7590						
Assay	**Specific Activity(*)**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	**Taq Inhibition**	**Functional Assay**
Units Tested	**n/a**	**50**	**50**	**50**	**n/a**	**n/a**
Specification	74,625 U/mg	<10.0% Released	<1.0% Released	No Conversion	Pass	Functional with buffers B7590 and B7591

* Taq DNA Polymerase (P7580)

Source of Protein: A recombinant E. coli strain carrying the Taq DNA polymerase gene from the thermophilic organism *Thermus Aquaticus* YT-1 complexed with a monoclonal antibody derived from murine cell culture.

Unit Definition: 1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

Quality Control Analysis:

Functionality of Phoenix Hot Start Taq DNA polymerase is evaluated by its ability to amplify DNA targets by PCR in reaction buffers B7590 and B7591 following a 24 hour incubation at room temperature. After heat activation and PCR amplification the samples resulted in visible single band amplicons as determined by agarose gel electrophoresis.

Taq Inhibition is measured by the residual activity of Phoenix Hot Start Taq DNA polymerase in the absence of a ≥ 94ºC heat activation step. Phoenix Hot Start Taq DNA polymerase and Taq DNA Polymerase (Taq DNA Polymerase without Taq Antibody) were incubated in the presence of Calf Thymus DNA, 50 µM 3H-dTTP, 100 µM dNTPs and 1X reaction buffer B7590 or B7591. After a 24 hour incubation at room temperature the samples were analyzed for total 3H-dTTP counts incorporated using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Supplied in: 20 mM Tris-HCl, 100mM NaCl, 0.1mM EDTA, Stabilizer 50% glycerol pH 7.5 @ 25ºC.

Supplied with: 5X Phoenix Hot Start Taq Reaction Buffer (B7590) and 5X Phoenix Hot Start Taq GC Reaction Buffer (B7591)

References:

1. Chou, Q. et al. (1992) Nucleic Acids Research 20(7), 1717-1723.
2. Sharkey, D. et al. (1994), Nature Biotechnology, 12, 506 – 509.

Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

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)

* Total reaction volume can be adjusted as needed

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.

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	50 – 250 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 Mg2+ in the final reaction (i.e. when diluted to 1X). In cases where additional Mg2+ optimization is required, adjust the final Mg2+ concentration in 0.2 mM steps.

Frequently Asked Questions and Troubleshooting

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

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