Phoenix™ Hot Start Taq DNA Polymerase

Key benefits

- Increased amplification specificity compared to regular Taq DNA polymerase
- Antibody hot start method for rapid reactivation and shorter PCR cycle time
- Reaction mix stability >72 hours at room temperature is optimized for automated workflows
- Simplified PCR optimization – tolerates a broad range of Mg^{2+} concentrations and annealing temperatures
- Increased amplification yield and overall success rate, minimizing rework time
- High sensitivity formulation with successful amplification down to 300 pg human gDNA input
- Demonstrated multiplexing capability with minimal optimization
- High success rates with GC-rich targets

High amplification specificity

PCR is routinely used for DNA amplification and serves several downstream applications, including cloning or DNA sequencing. Ensuring stringent conditions in PCR has become increasingly important, with fidelity playing a crucial role in ensuring accurate replication of DNA sequences and minimizing errors that could compromise the reliability of downstream applications, such as sequencing and synthetic biology.

Phoenix Hot Start Taq DNA Polymerase combines the purity of QIAGEN Taq B DNA polymerase with a proprietary antibody formulation that inhibits polymerase activity at room temperature. The 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. Activity of the Taq DNA polymerase is fully restored when the temperature of the PCR mixture reaches ≥94°C during the initial DNA denaturing step of PCR cycling (Figure 1).

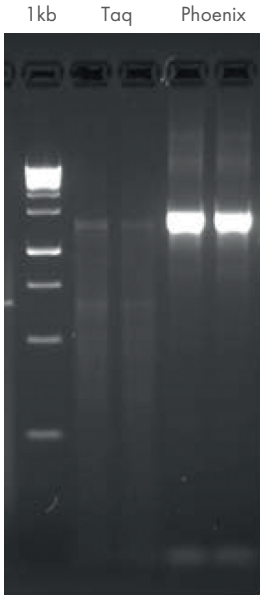


Figure 1
Improved specificity from the presence of the antibody hot start mechanism. PCR reactions were set up in-house at room temperature and preincubated for 24 hours prior to thermal cycling.

Rapid reactivation plus extended stability

Hot start polymerase products are divided into three categories, based on antibody, aptamer or chemical hot start mediation. Antibody methods have the advantage of rapid reactivation, minimizing reaction cycle times and increasing success rates. The weaker association of an antibody with the polymerase instantly yields larger units of active polymerase. Reactivation times for chemical methods are longer, often up to 10 minutes, but the chemical modification also allows reaction mixtures to remain inactive for multiple days. This feature enables automation and stacking of reactions, something that was previously unattainable using antibody-based methods.

Phoenix Hot Start Taq represents a major advance by combining rapid enzyme reactivation with extended reaction stability (>72 h) at room temperature.

Simplified reaction setup

The use of hot start methods has greatly simplified PCR reaction setup. Phoenix Hot Start Taq DNA Polymerase redefines simplicity, as shown in Figure 2, where assembled reaction mixtures with amplicons of increasing size and varying GC content are stable at room temperature for at least 72 hours with no impact on specificity or yield.

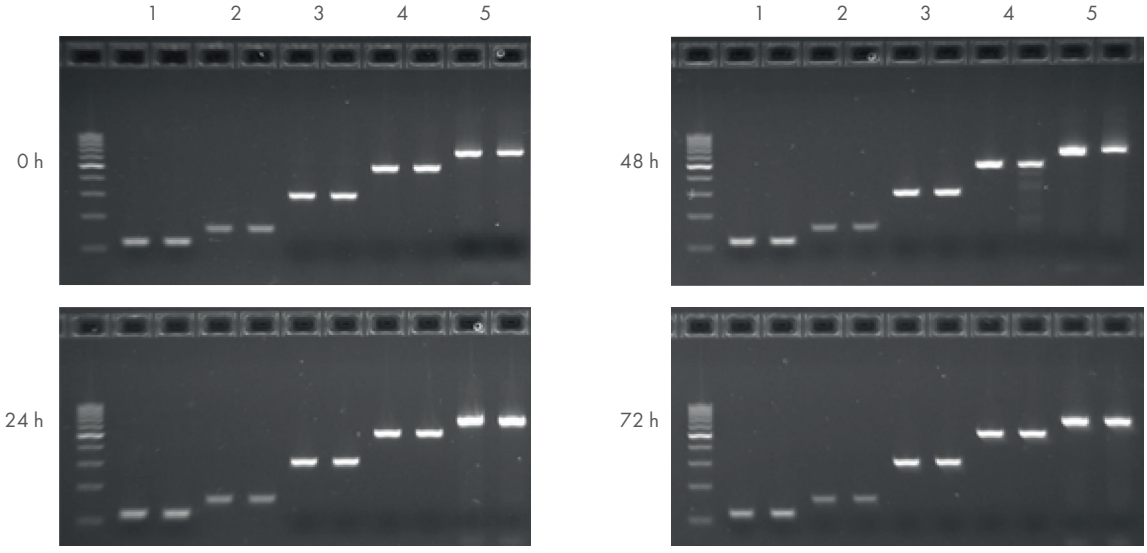
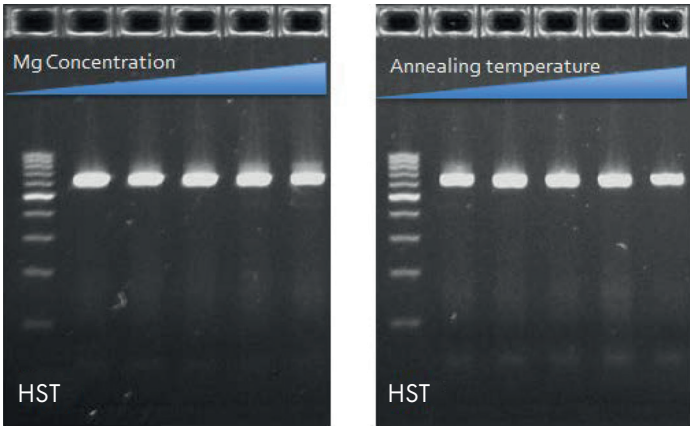


Figure 2
Successful amplification of a range of amplicons varying in size and GC content (50 ng human gDNA input) demonstrates that incubation time has no effect on yield or specificity.



Phoenix Hot Start Taq DNA Polymerase also tolerates a broad range of Mg^{2+} concentrations and a wide range of annealing temperatures (Figure 3).

Figure 3
Successful amplification of a 653 bp amplicon with increasing Mg^{2+} concentrations (2 mM to 3 mM in 0.25 mM increments; 58°C extension temperature) and varying annealing temperatures (56°to 64°C in 2°C increments; 2mM Mg^{2+} concentration).

Sensitivity

Higher stringency levels demand a formulation that will yield successful amplifications from precious samples. Phoenix Hot Start Taq DNA Polymerase is formulated from highly pure polymerase and antibodies and is capable of amplification from template quantities as low as 300 pg human gDNA (Figure 4).

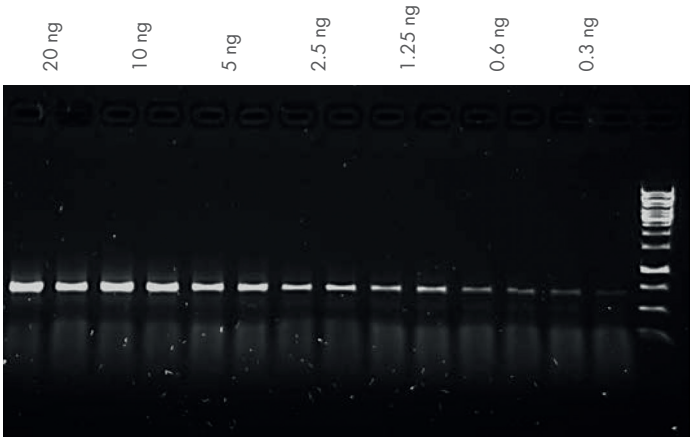


Figure 4
Presence of the desired 653 bp amplicons from 20 ng template to as low as 300 pg input material.

Multiplex amplification

Phoenix Hot Start Taq DNA Polymerase has demonstrated multiplexing capability. Minimal optimization is required for successful amplifications of targets varying in size and GC content (Figure 5).

Increased PCR success rate

To validate the performance of Phoenix Hot Start Taq DNA Polymerase, the formulation was tested in-house on an expanded panel of amplicons of varying length and GC complexity versus the industry leading antibody hot start DNA polymerase. A win was determined by the presence of a noticeably stronger band on a gel for a panel of 72 PCR reactions of varying amplicon size and GC content. The results obtained confirmed increased success rate, decreased failures and an improvement in specificity with Phoenix Hot Start Taq DNA Polymerase. An incremental increase in accurate amplification equates to a significant decrease in rework for failed reactions. Subsequent yield comparison showed that Phoenix Hot Start Taq had higher yields. Including Mg^{2+} in the formulation simplifies workflow optimization of Phoenix Hot Start DNA Polymerase. We recommend using 1 U per amplicon as a starting point in optimization experiments. Increasing the units of Phoenix Hot Start DNA Polymerase has yielded successful results on complex templates that were previously difficult to amplify.

	Target	Size (bp)	%GC
1	ABL1	101	58
2	BRCA	201	46
3	TP53	295	56
4	EGFR	397	47
5	BRCA1	501	40
6	TP53	599	56
7	BRCA2	703	33
8	BRCA1	803	40
9	ABL1	899	45
10	EGFR	999	40

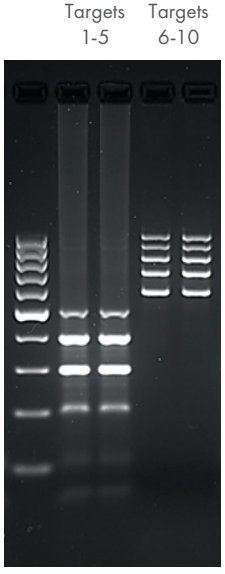


Figure 5
Result of two separate 5plex amplifications of human gDNA with Phoenix Hot Start Taq DNA Polymerase.

Amplification size range

In-house testing shows that Phoenix Hot Start Taq DNA Polymerase can amplify targets up to 4 kb more effectively than the competitor hot start formulation (Figure 6). If your amplification length requirements are greater than 2.5 kb, we recommend switching to VeraSeq™ 2.0 High Fidelity DNA Polymerase.

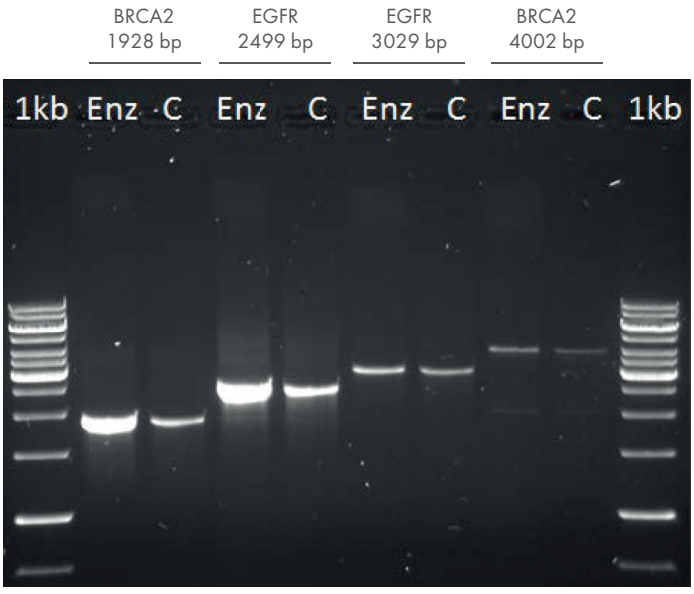


Figure 6
Improved performance of Phoenix Hot Start Taq DNA Polymerase (Enz) versus the competitor hot start formulation (C).

Quality and service you can count on

QIAGEN manufactures pure, superior quality enzymes and reagents for molecular biology and other applications. The company strives to resolve customers' challenges by providing high quality materials, a consistent supply chain and excellent service. With a manufacturing record unmatched in commercial enzyme production, QIAGEN designs analytical grade quality into all its products to meet the most rigorous specifications. Phoenix Hot Start Taq DNA Polymerase reflects our commitment to identifying, developing, and delivering outstanding enzyme technologies. If your company requires products and a service partner that stand above the crowd, we'd love to hear from you.

Ordering Information

Product	Contents	Cat. no.
Phoenix Hot Start Taq DNA Polymerase	500 U at 5000 U/mL	P7590L
VeraSeq 2.0 High-Fidelity DNA Polymerase	500 U at 2000 U/mL	P7511L

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