PRODUCT NOTE

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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.
- >72h room temperature reaction mix stability simplifying reaction set up and optimized for automated workflows.
- Simplified optimization – tolerates a broad Mg^{2+} and annealing temperature ranges, simplifying the PCR optimization.
- Increased amplification yield and overall success rate, minimizing rework time.
- High sensitivity formulation with successful amplification down to 300pg human genomic DNA input.
- Demonstrated multiplexing capability with minimal optimization.
- High success rates with GC rich targets

The PCR is routinely used for DNA amplification and serves a number of downstream applications, such as cloning or DNA sequencing. The requirements for the PCR continue to increase in stringency with amplification specificity of key importance. Phoenix™ Hot Start Taq DNA Polymerase is a combination of Enzymatics' purity leading Taq B DNA polymerase with a proprietary antibody formulation that inhibits polymerase activity at room temperature. Due to highly specific binding of the antibody inhibitor, Phoenix™ Hot Start Taq DNA polymerase is provided in an inactive form, allowing room temperature reaction set up, eliminating or reducing non-specific amplification at the start of the PCR. Rapid and complete reactivation of the polymerase activity (94°C for 2 minutes), coupled to Enzymatics' leading purity standards, results in increased sensitivity, specificity, and overall PCR success rate.

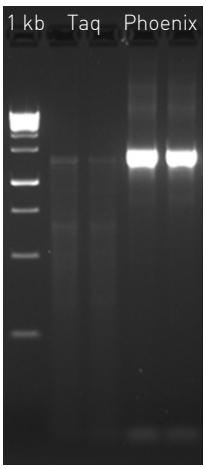

Figure 1.
Improved specificity from the presence of the antibody hot start mechanism. PCR reactions were set up at room temperature and preincubated for 24 h prior to thermal cycling.

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The Best of All Worlds

Hot start polymerase products have been split into three major segments, antibody, aptamer, and chemical hot start methods. Antibody methods are preferred for their rapid reactivation, minimizing reaction cycle times and increasing success rates as the weaker association of an antibody with the polymerase yields larger units of active polymerase instantly. Chemical methods have longer reactivation times, often up to 10 minutes. The chemical modification allows reaction mixtures to remain inactive for multiple days, enabling automation and stacking of reactions, something that up until now has been out of reach of antibody based methods. Phoenix™ Hot Start Taq is a paradigm shift with rapid reactivation and extended (>72h) room temperature reaction stability.

Simplified reaction set up

The use of hot start methods for the PCR greatly simplifies reaction set up. Phoenix™ Hot Start Taq DNA polymerase takes simplicity to a new level. Figure 2 highlights a truly innovative feature of the formulation; assembled reaction mixtures are stable at room temperature for at least 72 h with no impact on specificity or yield.

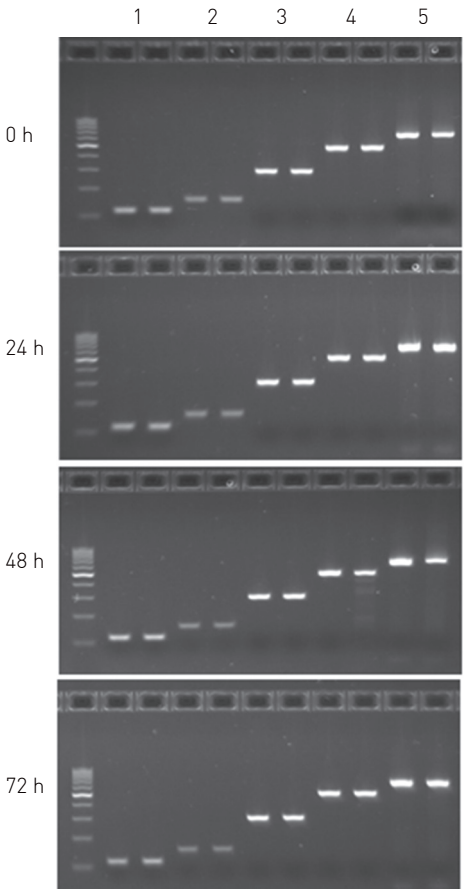

Figure 2. Successful amplification of a range of amplicons of increasing size and varying GC content (50 ng human gDNA input). Incubation times are shown to have zero impact on success rate.

Phoenix™ Hot Start Taq DNA polymerase has also been shown to tolerate a broad range of Mg^{2+} concentrations and a wide range of annealing temperatures (Figure 3).

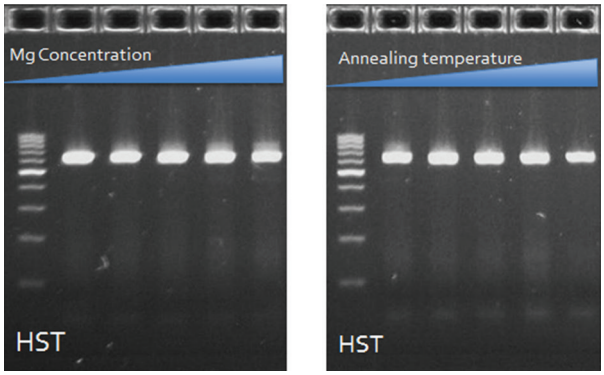

Figure 3. Successful amplification of a 653 bp amplicons with increasing Mg^{2+} concentrations (58oC extension temperature, 2 mM to 3 mM in 0.25mM increments) and varying annealing temperature from 56 to 64°C in 2°C increments. Mg^{2+} is held constant at 2mM during the annealing temperature experiments.

Sensitivity

As customer requirements from the PCR become more demanding and stringency is pushed to higher and higher levels, the need for a formulation that will yield successful amplifications from your precious samples continues to increase. Phoenix™ Hot Start Taq DNA polymerase is formulated from the highest purity polymerase and antibodies and this is capable of amplification from template quantities as low as 300 pg human gDNA.

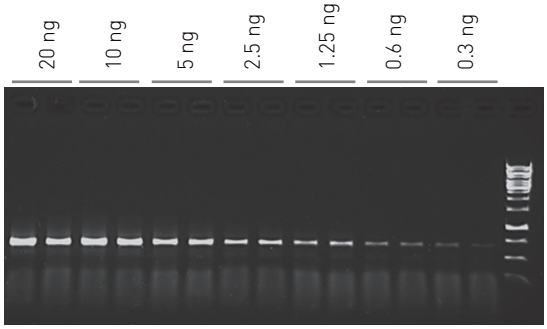

Figure 4. The presence of the desired 653bp amplicons down to as low as 300pg input material.

Multiplex amplification

Phoenix™ Hot Start Taq DNA polymerase readily drops into multiplexed experiments. With no optimization, the following multiplexing was observed:

	TARGET	SIZE (bp)	%GC
1	ABL1	101	58
2	BRCA2	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

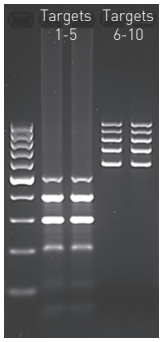

Figure 5. Shows two separate 5-plex amplifications.

Increased PCR success rate

To validate the performance of Phoenix™ Hot Start Taq DNA polymerase, the formulation was tested on an expanded panel of amplicons, of varying length and GC complexity versus the industry leading antibody hot start DNA polymerase. The results obtained confirmed increase success rate, decreased failures and an improvement in specificity. An incremental increase in perfect amplification equates to a significant decrease in rework for failed reactions. Subsequent yield comparison clearly demonstrated that Phoenix™ Hot Start Taq shows no compromise on specific amplifications and has demonstrably higher yields. A product format with Mg^{2+} included in the formulation ensures that optimization of Phoenix™ Hot Start DNA polymerase into your workflow is greatly simplifed. We are recommending the use of 1U per amplicon for a starting point in optimization experiments. The use of increasing amount of Phonenix™ Hot Start DNA polymerase units has shown to yield successful results on complex templates that previoulsy were not amplified.

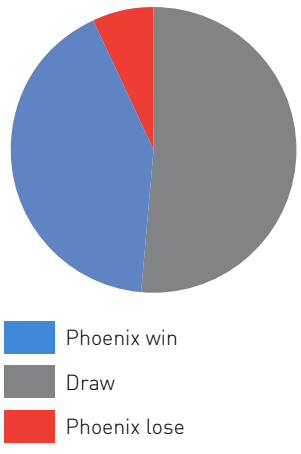

Figure 6. A summary view assessing the performance of Phoenix™ Hot Start Taq DNA Polymerase versus the industry leading antibody hot start formulation. A win is determined by the presence of a noticeably stronger band on a gel for a panel of 72 PCR reactions of varying amplicon size and G:C content.

Amplification size range

Phoenix™ Hot Start Taq DNA polymerase has been shown to amplify routinely up to 4kB more effectively than the competitor hot start formulation. If your amplification length requirements increase (>2.5kB) we recommend switching to our highest fidelity DNA polymerase offering, VeraSeq™ 2.0 High Fidelity DNA polymerase.

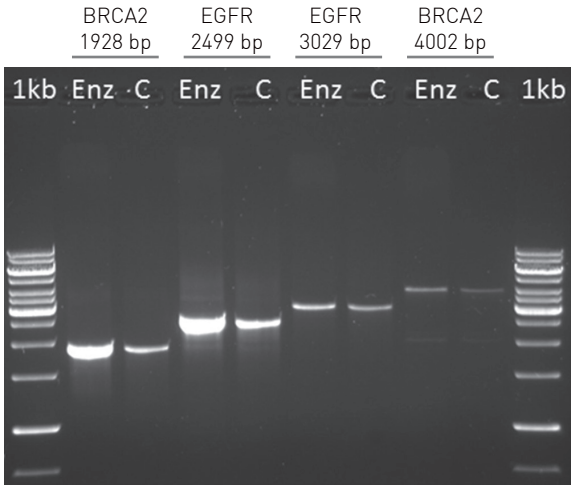

Figure 7. Improved performance of Phoenix™ Hot Start Taq DNA polymerase versus the competitor hot start formulation (C).

Quality and Service You Can Count On

Enzymatics manufactures pure, highest quality enzymes and reagents for molecular biology and other applications. Founded in 2006 by expert enzyme production scientists, Enzymatics has strived to resolve customers' challenges by providing the highest-quality materials available, an unbreakable supply chain, and paradigm-shifting service. With a manufacturing record unmatched in commercial enzyme production, Enzymatics designs analytical grade quality into all its products to meet the most rigorous specifications.

The introduction of our new, Phoenix™ Hot Start Taq DNA polymerase furthers our commitment to identifying, developing, and delivering the very best protein technologies. If your company has a requirement for a protein product and a service partner that stands above the crowd then we want to hear from you.

ORDER DETAILS			
Product Description	Part #	Concentration	Unit Size
Phoenix™ Hot Start Taq DNA Polymerase	P7590L	5000U/mL	500

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