



# CleanAmp™ dNTPs

**The Next Generation in Hot Start PCR**

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## Application Note



**TriLink**  
BioTechnologies

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## Introduction

As a diagnostic tool in molecular biology, the Polymerase Chain Reaction (PCR) is one of the most powerful and commonly used techniques. However, inherent flaws can plague PCR, especially when nonspecific amplification such as primer dimer formation dominates the reaction. These off-target amplifications are due to the excess of primers in each experiment, where primer interaction and extension are inevitable (1). Competing off-target amplifications decrease the overall effectiveness of PCR by consuming key components within the reaction and inhibiting the amplification of the intended target (2).

Commonly used approaches to mitigate the formation of off-target amplification in PCR are termed Hot Start (3). In this process, the components of a PCR reaction are prevented from forming primer extension products until higher thermocycling temperatures are reached. Many developments have been made in this technology, including strategies that require the physical separation of reaction components, inhibition of DNA polymerase, the addition of accessory proteins and the use of chemically modified primers that are unextendable (2,3). While these modifications to PCR setup can be effective, they can also significantly increase the cost of reactions. One area that had not been explored is the concept of deoxynucleoside-5'-triphosphates (dNTPs) that are modified to allow for a Hot Start activation approach in PCR. Since dNTPs are ubiquitous in all PCR experiments, modified dNTPs can easily be incorporated in any reaction by simple substitution. CleanAmp™ dNTPs provide a novel, cost-effective and efficient route to Hot Start PCR that is amendable to use in standard PCR protocols, with a wide range of commonly-used DNA polymerases.

CleanAmp™ dNTPs contain thermolabile modification groups that allow for dNTP-mediated Hot Start activation in PCR. The introduction of temperature sensitive tetrahydrofuryl protecting groups onto the 3'-hydroxyl of a dNTP blocks primer extension at

the less stringent, lower temperatures of PCR reaction preparation (Figure 1). When the reaction is heated to the elevated temperatures of PCR, the protecting group is removed to form the corresponding standard dNTP, which is now a suitable DNA polymerase substrate. The loss of protecting group can be attributed to the acidification of Tris-based PCR buffers as temperature is increased (for example, an increase in temperature will cause a Tris-based reaction buffer at ~ pH 8 (25°C) to become ~ pH 6 at elevated temperatures (95°C)). This temperature-dependent control of dNTP activation has shown great promise for the use of CleanAmp™ dNTPs with DNA polymerases that utilize Tris-based PCR buffers, pH 8 to 9 at 25°C.

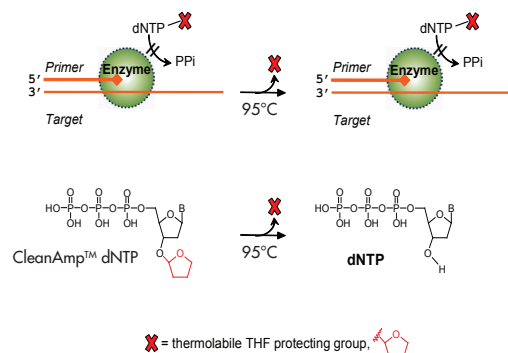


Figure 1: Hot Start Activation of CleanAmp™ dNTPs.

The efficacy of CleanAmp™ dNTPs is highlighted herein as they are able to decrease the formation of primer dimer products while increasing the specificity of the desired product. The following experiments explore the effectiveness of CleanAmp™ dNTPs in primer/template systems prone to primer dimer artifacts by comparing their performance to unmodified dNTPs and Hot Start DNA polymerases. Studies will also demonstrate the compatibility of CleanAmp™ dNTPs when used in combination with a variety of non-Hot Start and Hot Start DNA polymerases.

## Eliminate Primer Dimer Formation, Increase Yield

CleanAmp™ dNTP modifications significantly lower or eliminate primer dimer formation while increasing target amplicon yield in endpoint PCR. A problematic 533 bp primer/template system from Lambda genomic DNA is prone to primer dimer formation in PCR, especially at low template concentrations (4). After 40 thermal cycles, reactions that employed natural dNTPs are compromised by competing amplification of non-target sequences (Figure 2). Under standard PCR conditions, the effect on specificity and sensitivity of detection is most significant at lower template concentrations as the target amplicon is completely absent and primer dimers predominate. When CleanAmp™ dNTPs are substituted for standard dNTPs, not only are primer dimers significantly reduced, the desired amplicon's yield is increased as well. By introducing CleanAmp™ dNTPs into a PCR setup, primer dimers and other off-target amplicons are significantly reduced, resulting in a cleaner PCR reaction. Although these results were obtained using 0.2 mM concentration, the most consistent results are seen with a dNTP concentration of 0.4 mM and a MgCl<sub>2</sub> concentration of 2.5 mM.

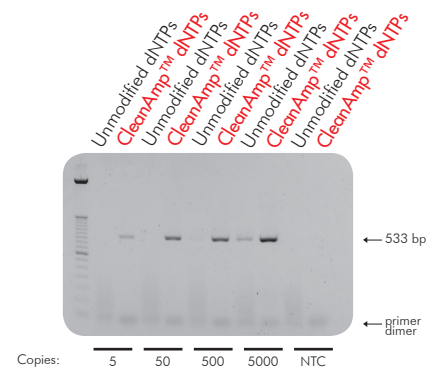


Figure 2: Endpoint PCR evaluation of CleanAmp™ dNTPs in a primer/template system prone to primer dimer. PCR Conditions<sup>1</sup> on page 10.

## Improve Specificity Over a Greater Range of Template Concentrations

CleanAmp™ dNTPs provide amplification specificity over a greater range of template concentrations in real-time PCR. Real-time detection of the above-mentioned 533 bp target displays reduced sensitivity in real-time detection due to predominant primer dimer formation at lower template concentrations. The detection of the Lambda gDNA amplicon was tested over a variety of template concentrations ranging from 5-5,000 copies using TaqMan® probe detection in real-time PCR (Figure 3). When 5 to 50 copies of template were employed, the use of standard dNTPs failed to amplify the target band, while CleanAmp™ dNTPs successfully yielded the desired product at all template concentrations. When the resulting data was plotted in a standard curve, CleanAmp™ dNTPs afforded a 10-100 fold increase in sensitivity relative to standard dNTPs, thus allowing for detection over a wider range of template concentrations. By increasing the limit of detection, CleanAmp™ dNTPs give the user more options and fewer restrictions in experimental design for real-time target detection.

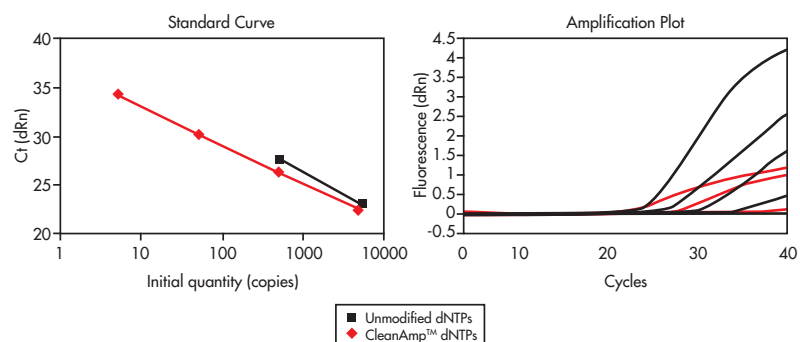


Figure 3: Real-time PCR detection of 5-5,000 copies of Lambda gDNA using CleanAmp™ dNTPs. PCR Conditions<sup>1</sup> on page 10.

## Increase PCR Reproducibility

Real-time PCR results often vary and require multiple iterations to achieve reproducible data, especially when template concentrations are limiting (3,5). With CleanAmp™ dNTPs, PCR data fluctuates very little within an experimental run, allowing analysis with confidence. In the real-time experiment shown in Figure 4, CleanAmp™ dNTPs were tested against unmodified dNTPs at different template concentrations in two separate primer/template systems. For both the HIV-1 and Lambda DNA targets, CleanAmp™ dNTPs provide greater experimental precision, especially at lower template concentrations. The standard deviation at these low concentrations is high under standard conditions, yet decreases considerably when CleanAmp™ dNTPs are used in the reaction. Even at high template concentrations, where the standard deviation of reactions with standard dNTPs is low, CleanAmp™ dNTPs still produce a more consistent result. With CleanAmp™ dNTPs, more time is spent analyzing data instead of second-guessing PCR results.

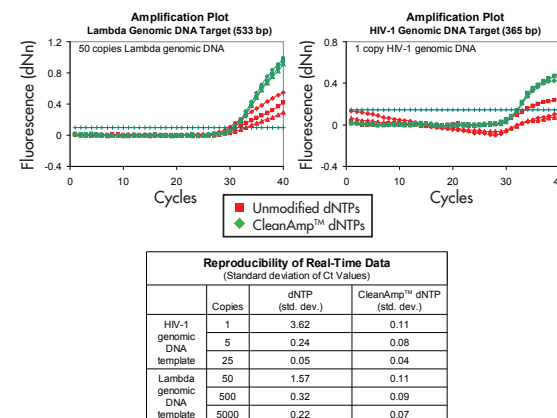


Figure 4: SYBR green real-time PCR assay where results of 1 copy of HIV-1 gDNA and 50 copies of Lambda gDNA were consistent when using CleanAmp™ dNTPs. A. Amplification plots of the real-time data. B. Real-time data in chart form. PCR Conditions<sup>1,2</sup> on page 10.

## Versatility of CleanAmp™ dNTPs

Though *Taq* is one of the most commonly used DNA polymerases in PCR, there are other thermostable DNA polymerases that may be amenable for use with CleanAmp™ dNTPs. To investigate further, seven non-Hot Start DNA polymerases were tested in PCR with endpoint agarose gel analysis to learn whether they could successfully amplify a 365 bp target band with either standard or CleanAmp™ dNTPs (Figure 5). When using standard dNTPs, Deep Vent<sub>R</sub>™ amplified only primer dimer while *Tth* and *Tfi* DNA showed only slight amplification. Furthermore, off-target amplification is evident for most of the remaining DNA polymerases. In contrast, the introduction of CleanAmp™ dNTPs into these experiments eliminated or reduced primer dimer formations, and all of the DNA polymerases were able to produce the desired 365 bp amplicon. CleanAmp™ dNTPs work well together with a broad selection of DNA polymerases, in addition to *Taq* DNA polymerase, further demonstrating their versatility.

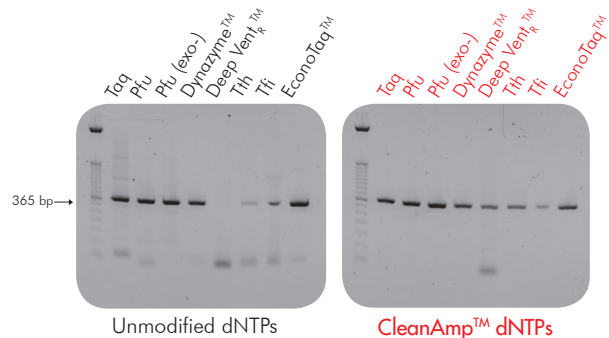


Figure 5: Evaluation of the performance of CleanAmp™ Primers in amplification reactions with a variety of thermostable DNA polymerases. PCR Conditions<sup>2</sup> on page 10.

## Achieve Better Results Compared to Hot Start DNA Polymerases

In these experiments, reactions containing *Taq* DNA polymerase with CleanAmp™ dNTPs were compared to reactions with various Hot Start DNA polymerases and natural dNTPs for their ability to amplify a 365 bp target from HIV-1 (Figure 6) (3). When tested against other common Hot Start technologies, CleanAmp™ dNTPs provided a comparable reduction in primer dimer formation. Although all Hot Start approaches successfully suppressed off-target amplicon formation in this primer/template system, reactions containing *Taq* DNA polymerase and CleanAmp™ dNTPs outperformed the Hot Start technologies by providing a greater relative amplicon yield. Overall, when evaluated alongside other Hot Start technologies, CleanAmp™ dNTPs display comparable off-target results while enhancing target amplification. Other primer/template systems may require further optimization. See the CleanAmp™ dNTP Product Manual for specific details.

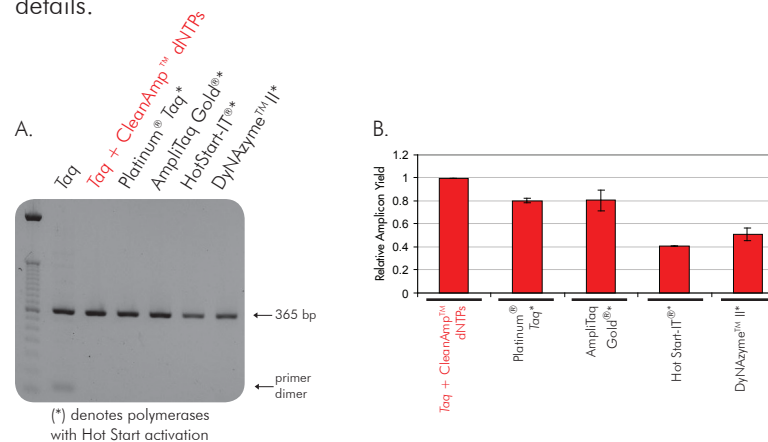


Figure 6: Comparison of CleanAmp™ dNTPs to other commercially available Hot Start DNA polymerases. A. Endpoint PCR analysis of amplification reactions containing 5 copies of HIV-1 genomic DNA. B. Graphical representation of the relative amplicon yield (normalized to *Taq* + CleanAmp™ dNTPs). PCR Conditions<sup>2</sup> on page 10.

## Improve Performance with Other Hot Start Technologies

When used in combination with other Hot Start technologies, CleanAmp™ dNTPs allow for an even greater enhancement in PCR specificity. In Figure 7, experiments were conducted with a Lambda gDNA template system, known for its susceptibility to primer dimer formation. When Platinum® Taq and HotStart-IT® Taq were used with standard dNTPs, relative amplicon yield improved compared to Taq DNA polymerase, but primer dimer formation was still evident. With the addition of CleanAmp™ dNTPs, off-target amplification is significantly reduced or eliminated. Similarly, TriLink's CleanAmp™ Turbo and Precision Primers both increased relative amplicon yield, yet had some primer dimer byproducts. When used in conjunction with CleanAmp™ dNTPs, an improvement in PCR performance was seen, especially with CleanAmp™ Precision Primers, where off-target amplification was entirely eliminated. When used individually, each Hot Start approach improves the specificity and efficiency of PCR reactions, but this improvement is further enhanced when the Hot Start technologies are used in combination with CleanAmp™ dNTPs.

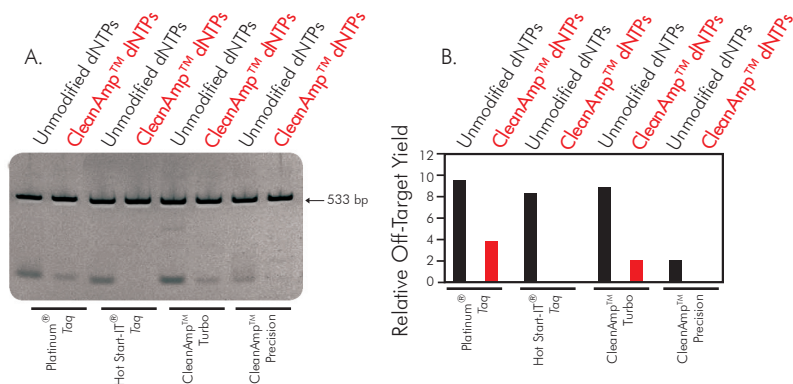


Figure 7: Comparison of commercially available Hot Start DNA polymerases used with and without CleanAmp™ dNTPs. A. Endpoint PCR analysis of amplification reactions containing 5 copies of HIV-1 genomic DNA. B. Graphical representation of the relative off-target amplification. PCR Conditions<sup>1</sup> on page 10.

## Conclusions

### CleanAmp™ dNTPs:

- Present a novel Hot Start technology which reduces or eliminates primer dimer formation and mis-priming while enhancing target yield.
- Increase the limit of detection 10-100 fold relative to traditional PCR protocols.
- Provide comparable or better performance than Hot Start DNA polymerases at a fraction of the price.
- Enrich PCR specificity with a variety of thermostable DNA polymerases.
- Increase the reproducibility of PCR results within each experiment.
- Further improve the performance of other Hot Start DNA polymerases.

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## References

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## PCR Conditions

1. PCR: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl<sub>2</sub>), Primers (0.2 μM), 0.2 mM dNTPs, 5,000 copies Lambda gDNA, 1.25 U Taq DNA polymerase, 50 μL. Thermal cycling: 95°C (10 min); [95°C (40 sec), 57°C (30 sec), 72°C (60 sec)] 40X.
2. PCR: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl<sub>2</sub>), Primers (0.4 μM) 0.2 mM dNTPs, 5 copies HIV-1 gDNA, DNA polymerase (var. U), 50 μL. Thermal cycling: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2min)] 35X. 72°C (7min).

NOTE: TaqMan® probe (0.1 μM) and ROX were used for real-time experiments.

## Contact Information

For further information on CleanAmp™ dNTPs, please visit our website or contact TriLink directly:

### CleanAmp™ Products online:

[www.trilinkbiotech.com/cleanamp](http://www.trilinkbiotech.com/cleanamp)

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