



# CLEANAMP™ PCR PRODUCTS

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

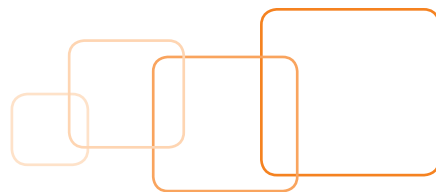
 **TriLink**  
BioTechnologies  
The Modified Nucleic Acid Experts

# CleanAmp™ Products

## The Next Generation in Hot Start PCR

The CleanAmp™ line of products are TriLink's innovative new approach to Hot Start PCR. We applied our expertise in modified nucleic acid chemistry to develop cost-effective solutions to common PCR problems. This advancement in Hot Start technology employs two widely ignored components of the PCR reagent mix: the primers and the dNTPs. Two distinct types of nucleic acid thermolabile protecting groups were identified by TriLink's R&D team to make the CleanAmp™ technology possible.

- Reduce or eliminate off-target amplification
- Increase amplicon yield and specificity
- Commercialize for less with reasonable licensing options



### How to Order

CleanAmp™ Products may be ordered online, via email, fax or phone. Please inquire for unique formulations and bulk quantities. We accept payment by purchase order, wire transfer and credit card (MasterCard, Visa and American Express). Visit our website for new products and technical resources.

Phone 800-863-6801

Fax 858-546-0020

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

Email [sales@trilinkbiotech.com](mailto:sales@trilinkbiotech.com)

### Expert Technical Support

The TriLink Team is committed to fully supporting TriLink's products and services from the initial customer inquiry to post delivery technical assistance. The answers to many common questions can be found online in our FAQ database. Have a unique question? Visit our *Ask An Expert* blog. Our technical support team is also available to speak with you Mon-Fri, 7 am to 5 pm PT.

### Resources

TriLink offers a number of technical resources to aid in your research efforts. The following technical resources are available at [www.trilinkbiotech.com](http://www.trilinkbiotech.com).

- *Ask An Expert* blog
- Product line bibliography
- Publications
- FAQ database
- Literature corner
- Technical Articles

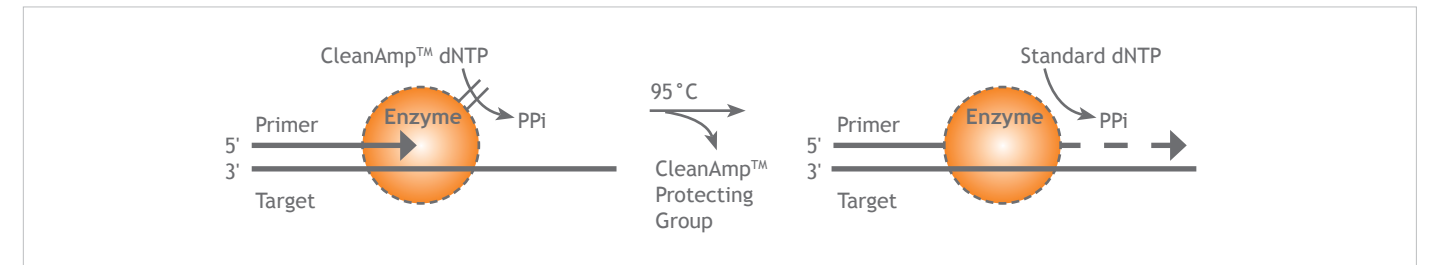
### ResearchRewards Program

TriLink offers ResearchRewards to help offset the cost of CleanAmp™ Primers, dNTPs and Amidites, as well as other nucleic acid-based products to support educators and researchers. To apply, email a completed application to [info@trilinkbiotech.com](mailto:info@trilinkbiotech.com). Applications may be submitted anytime. Applicants will be notified of the review results within two months.

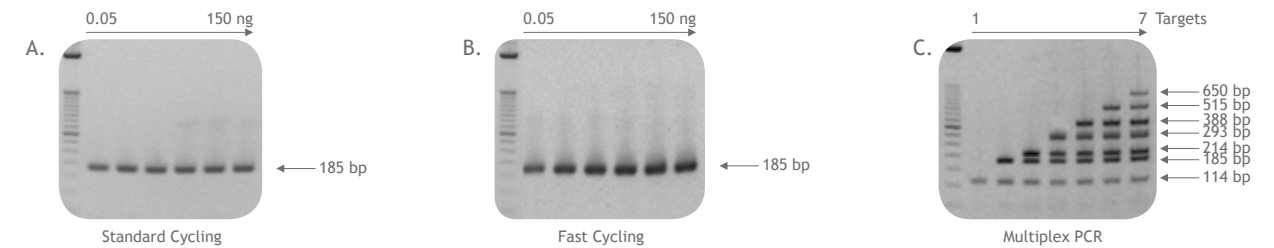
To download an application or learn more visit: [www.trilinkbiotech.com/about/researchrewards.asp](http://www.trilinkbiotech.com/about/researchrewards.asp)

## Introducing CleanAmp™ PCR Kits

CleanAmp™ PCR Kits are powered by TriLink's novel, heat-activated dNTP chemistry. Activation of the CleanAmp™ dNTPs occurs during the initial denaturation of Hot Start PCR and each subsequent thermal cycle, releasing just enough reagent to allow efficient amplification. By limiting the amount of active reagent during the early cycles when the target is in low concentration, CleanAmp™ significantly reduces background amplification to achieve a reduction or even an elimination of primer dimer and mis-priming.



CleanAmp™ PCR Kits benefit basic applications as well as more advanced variants, such as real-time, multiplex and fast PCR. Figure 1 demonstrates the CleanAmp™ PCR Kits' versatility. CleanAmp™ PCR Kits benefit fast cycling and multiplex PCR protocols in addition to standard cycling, even at low template concentrations.



**Figure 1:** CleanAmp™ PCR Kit has the flexibility for use in a variety of reaction formats including (A) standard and (B) fast thermal cycling protocols as well as in (C) multiplex PCR.

Product	Catalog #	Pack Size	Price
CleanAmp™ PCR Kit CleanAmp™ dNTP Mix (N-9506) (10 mM), <i>Taq</i> DNA Polymerase (5 units/μL), 10X PCR Buffer and 50 mM MgCl <sub>2</sub>	L-3201-200	200 rxns*	\$180

\*The PCR kit provides sufficient material for approximately 200, 25 μl reactions using our Standard Thermal Cycling Protocol.

## Customer Testimonials

“Using CleanAmp™ dNTPs in your PCR is an interesting way to improve specificity. This novel Hot Start method is easy to incorporate into any PCR master mix.”

- Carl Wittwer, M.D., Ph.D., Professor of Pathology at the University of Utah and co-founder of Idaho Technology

“CleanAmp™ dNTPs used with native *Taq* polymerase perform to specification in our demanding multiplex Scorpions™ assays at a substantial cost savings.”

- Richard DeScenzo, Ph.D., Microbiology Group Leader at ETS Laboratories

“CleanAmp™ dNTPs is a clever alternative to conventional antibody based Hot Start approaches that circumvents some of their limitations. We are very excited including it in our tools portfolio and expect to use it when developing delicate assays.”

- Mikael Kubista, Ph.D., CEO and founder of TATAA Biocenter

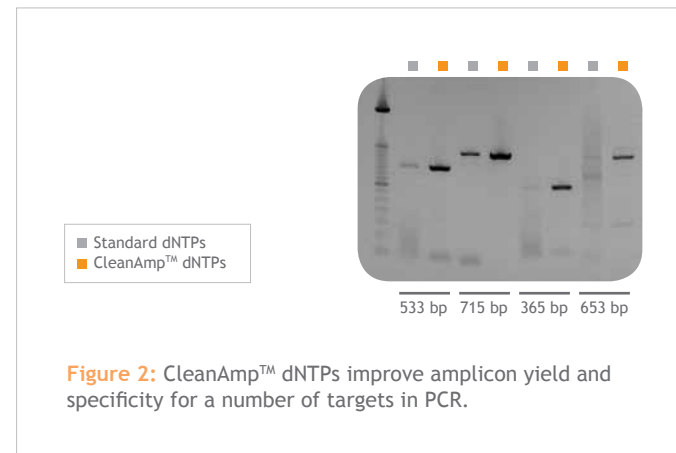
## CleanAmp™ dNTPs

### Make Any Assay a Hot Start

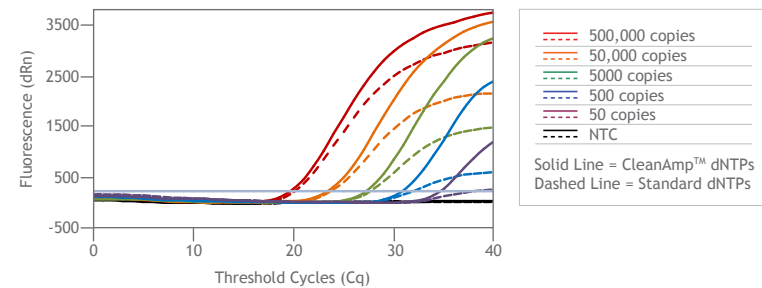
CleanAmp™ dNTPs, the key component in the CleanAmp™ PCR Kit, are also available separately. They are the most versatile solution in TriLink's line of PCR enhancing products, offering a universal approach to improved Hot Start PCR. Replacement of the standard dNTPs, the essential DNA polymerase substrate, with CleanAmp™ dNTPs, offers the same advantages as more costly Hot Start enzymes.

CleanAmp™ dNTPs offer precise control at the start of PCR thermal cycling by blocking nucleotide incorporation until heat activation of the dNTPs. The temperature-dependent control of DNA polymerase extension vastly reduces mis-priming, primer dimer formation and other common deleterious effects (Figure 2).

CleanAmp™ dNTPs improve in the specificity of target amplification, allowing for enhanced detection of low concentrations of template as shown in Figure 3. When standard dNTPs are employed reduced sensitivity in real-time detection is seen due to predominant primer dimer formation at lower template concentrations.



**Figure 2:** CleanAmp™ dNTPs improve amplicon yield and specificity for a number of targets in PCR.



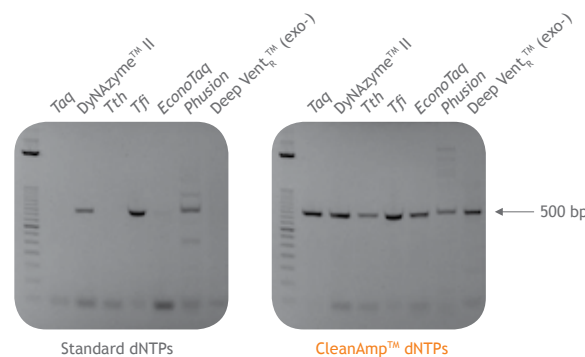
**Figure 3:** CleanAmp™ dNTPs improve the efficiency of PCR and increase the limit of detection in real-time PCR.

“Combining CleanAmp™ dNTPs with our novel lyophilization methods creates the ultimate in high quality easy-to-use PCR assays. Inclusion of CleanAmp™ dNTPs allows us to create accurate, reproducible and simple diagnostic kits, that greatly improve the quality of clinical testing and patient care.”  
- Mike Bunce, Ph.D., CEO and CSO at Biofortuna

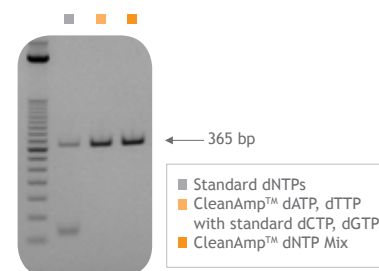
### A Flexible Solution

CleanAmp™ dNTPs improve the performance of a number of thermostable DNA polymerases, allowing for greater flexibility in PCR design. The use of CleanAmp™ dNTPs in the experiment shown in Figure 4 eliminated or reduced primer dimer formation in all cases. Moreover, each DNA polymerase was able to produce the desired amplicon in higher yield, demonstrating the versatility of CleanAmp™ dNTPs.

CleanAmp™ dNTPs are available in sets as well as in a mix, providing more flexibility in your experiment design, including the ability to achieve a Hot Start reaction without the need to substitute all four dNTPs. In Figure 5, a reduction in primer dimer formation and improvement in amplicon yield is achieved by substituting only dATP and dTTP with CleanAmp™ dATP and CleanAmp™ dTTP, respectively.



**Figure 4:** CleanAmp™ dNTPs are compatible with a number of commercially available DNA polymerases suited for PCR.



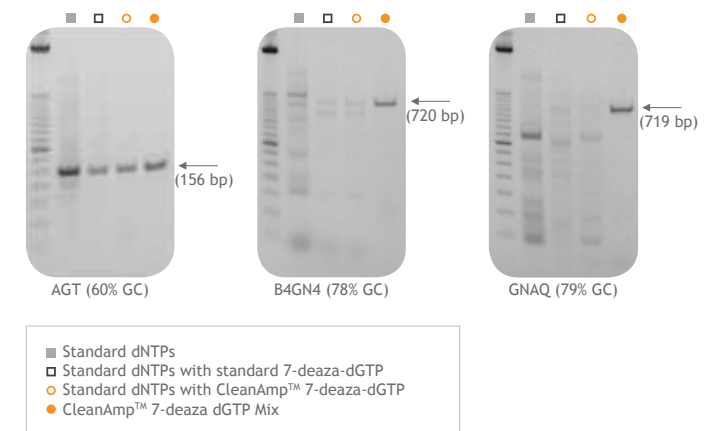
**Figure 5:** The CleanAmp™ dNTP Mix eliminates primer dimer formation, though significant improvements are also observed by substitution of only two nucleotides.

## CleanAmp™ 7-deaza-dGTP

### Robust Amplification of GC-Rich Targets

Struggling with amplification of DNA targets high in GC content? PCR product formation can often be compromised by inadequate strand separation and the propensity for complex secondary structure formation. The use of standard 7-deaza-dGTP is a notable method for overcoming this problem.<sup>1</sup> Recently, TriLink developed CleanAmp™ 7-deaza-dGTP, an elegant fusion of the secondary structure reducing nucleotide analog 7-deaza-dGTP and TriLink's CleanAmp™ dNTP Hot Start technology.

As depicted in Figure 6, when targets with increasing GC content are amplified, the use of the CleanAmp™ 7-deaza-dGTP Mix consistently provides a clean, high-yield product. For the 79% GC-rich GNAQ target, it is not possible to form the desired amplicon without the use of the CleanAmp™ 7-deaza-dGTP Mix. CleanAmp™ 7-deaza-dGTP is available individually for amplification of routine GC-rich targets or as the CleanAmp™ 7-deaza-dGTP Mix which is recommended for more challenging targets with higher GC content.



**Figure 6:** CleanAmp™ 7-deaza-dGTP Mix improves specificity for GC-rich target amplifications.

<sup>1</sup> McConlogue, L., Brow, M.A. and Innis, M.A. (1988) Structure independent DNA amplification by PCR using 7-deaza-2'-deoxyguanosine. Nucleic Acids Res, 16, 9869.

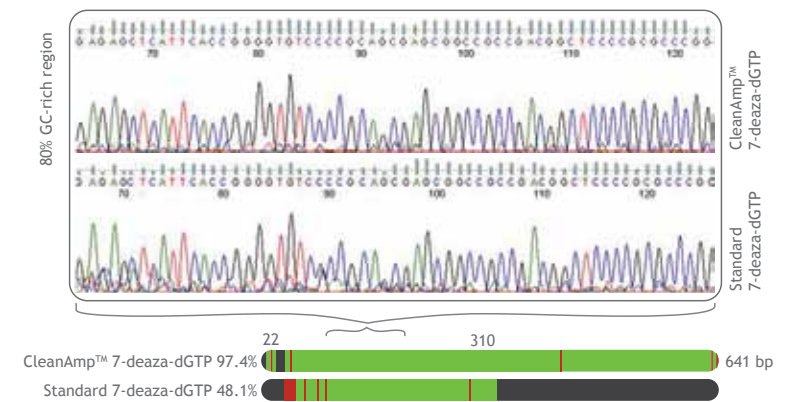
“I was having a terrible time trying to PCR amplify a region of DNA that is 62% G+C rich. Your new product, CleanAmp™ 7-deaza-dGTP Mix, made it a breeze and solved my problem. I have since recommended this to our collaborators and it is now an essential component in our protocol.”  
- Jane Michalski, University of Maryland, School of Medicine

### Improving Downstream Sequencing

GC-rich DNA targets can be difficult to sequence due to the high degree of intrastrand secondary structure formation. Sequencing GC-rich regions can also be complicated by loss of signal, band compressions and weak signal base calls. Pre-amplification of these problematic targets using CleanAmp™ 7-deaza-dGTP prior to sequencing can significantly improve the read quality. Complete substitution of CleanAmp™ 7-deaza-dGTP for dGTP in the pre-PCR step affords the best sequencing results.

As shown in Figure 7, even with the addition of standard 7-deaza-dGTP into the pre-amplification reaction, this troublesome 641 bp target cannot be sequenced past 310 bp. In comparison, the introduction of CleanAmp™ 7-deaza-dGTP nearly doubles the number of correct base calls.

The detailed sequence read shows a 60 bp stretch in which samples pre-amplified with standard 7-deaza-dGTP performed the strongest. In this 80% GC-rich stretch, reactions employing CleanAmp™ 7-deaza-dGTP substantially improved base call quality compared to reactions with standard 7-deaza-dGTP.



**Figure 7:** The use of CleanAmp™ 7-deaza-dGTP in a pre-sequencing PCR step improves the quality of base calling and length of reads in downstream sequencing reactions.

Product	Catalog #	Pack Size	Price
CleanAmp™ dNTP Mix dATP, dCTP, dGTP and dTTP each at 10 mM	N-9506-2 N-9506-10	2 μmoles each (4 x 2 μmoles)* 10 μmoles each (4 x 10 μmoles)	\$90 \$350
CleanAmp™ dNTP Set 1 (50 mM) Vial each of dATP, dCTP, dGTP and dTTP	N-9507-2 N-9507-10	2 μmoles each (4 x 2 μmoles)* 10 μmoles each (4 x 10 μmoles)	\$80 \$295
CleanAmp™ PCR Kit CleanAmp™ dNTP Mix (N-9506) (10 mM), Taq DNA Polymerase (5 units/μL), 10X PCR Buffer and 50 mM MgCl <sub>2</sub>	L-3201-200	200 rxns**	\$180
CleanAmp™ 7-deaza-dGTP Mix dATP, dCTP, (dGTP: 7-deaza-dGTP) and dTTP each at 10 mM	N-9504-2 N-9504-10	2 μmoles each of d(A,C,G***,T)TP* 10 μmoles each of d(A,C,G***,T)TP	\$120 \$465
CleanAmp™ 7-deaza-dGTP 50 mM	N-9515-2 N-9515-10	2 μmoles* 10 μmoles	\$120 \$465

\*A 2 μmole pack size is sufficient for approximately 200, 25 μl reactions at a 0.4 mM concentration or 400, 25 μl reactions at a 0.2 mM concentration.

\*\*The PCR kit provides sufficient material for approximately 200, 25 μl reactions using our Standard Thermal Cycling Protocol.

\*\*\*The CleanAmp™ 7-deaza-dGTP Mix contains a 1:3 ratio of dGTP:7-deaza-dGTP.

# CleanAmp™ Primers

## A Hot Start Assay for Less

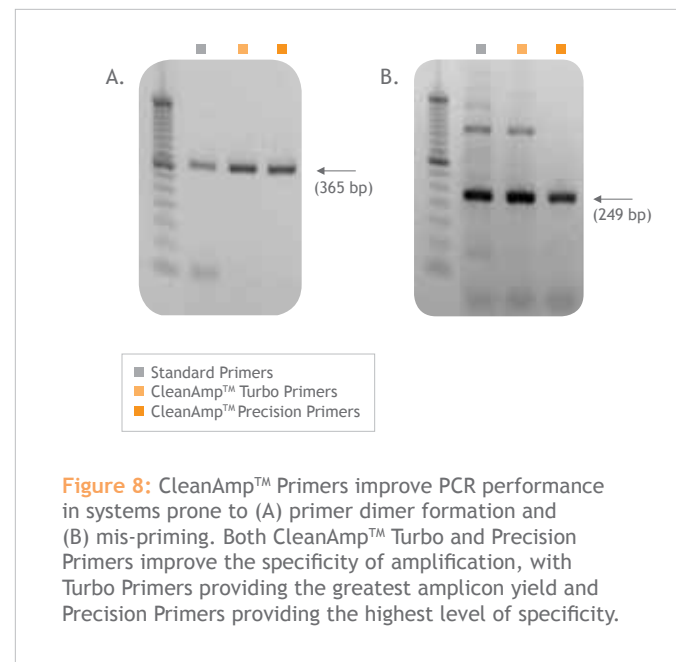
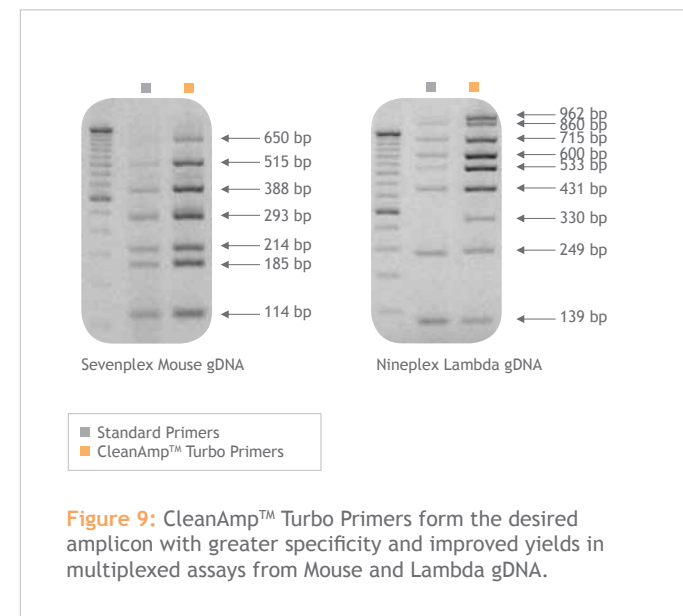
CleanAmp™ Primers are similar to CleanAmp™ dNTPs in that they contain thermolabile chemical modifications that allow for primer-based Hot Start activation in PCR. Like CleanAmp™ dNTPs, these modifications also serve to prevent primer extension at the lower temperatures of PCR setup and manipulation. A Hot Start thermal activation step removes the modification generating the corresponding unmodified primer, which supports amplification of the desired target. CleanAmp™ Primers specifically amplify your target by eliminating formation of primer dimer (Figure 8A) and mis-priming (Figure 8B) events. Furthermore, like CleanAmp™ dNTPs, CleanAmp™ Primers eliminate the need for Hot Start DNA polymerases because they are compatible with a number of standard DNA polymerases, such as *Taq*.

CleanAmp™ Primers are a key choice for diagnostic kits and other established assays. In these assays CleanAmp™ Primers can be as little as \$0.03 per reaction at our standard starting scale. TriLink guarantees at least 10 ODs per primer synthesis, which is enough material for approximately 6,250 reactions. TriLink offers two types of CleanAmp™ Primers that vary in the rate of thermal activation: Turbo and Precision. The different rates of release of CleanAmp™ Turbo and Precision Primer modifications can be exploited for improved results in advanced applications, such as multiplex PCR (Figure 9), reverse-transcription PCR, low copy number detection (Figure 10) and fast PCR. The performance of CleanAmp™ Primers can be applied to critical applications such as molecular diagnostics, forensics, detection of infectious agents and gene expression validation.

## Maximize Your Multiplex

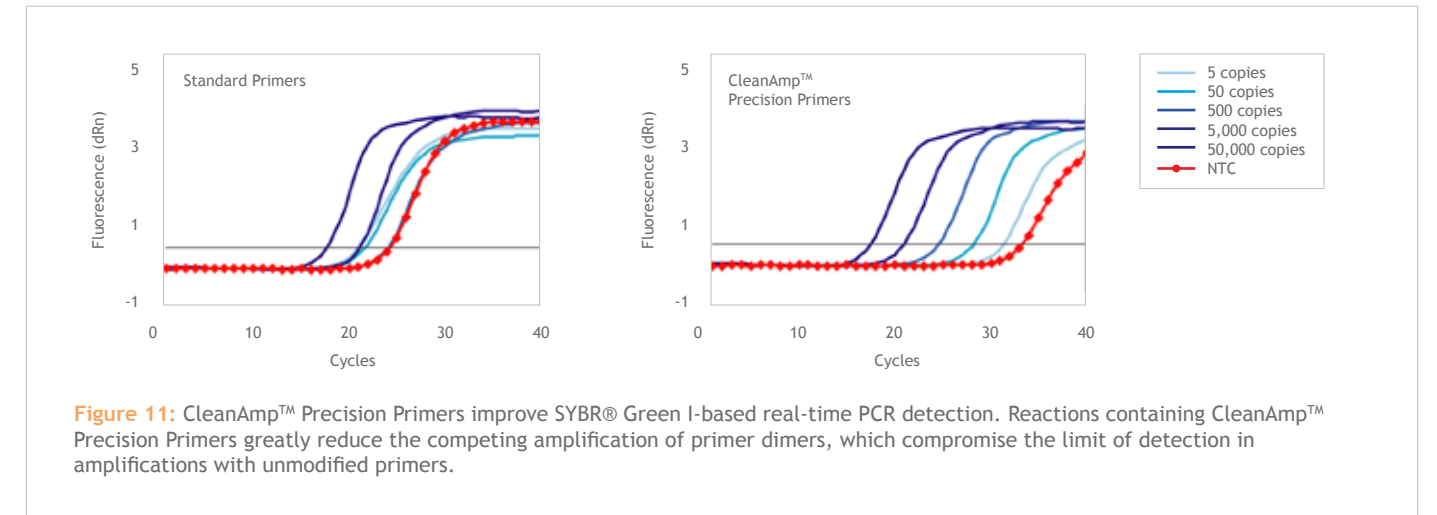
If you are seeking a Hot Start solution for a problematic multiplex system, CleanAmp™ Primers may be your answer. Like CleanAmp™ dNTPs, CleanAmp™ Primers will improve yield and specificity in multiplex systems. However, CleanAmp™ Primers offer higher specificity with a greater number of targets. In the systems shown in Figure 9, CleanAmp™ Turbo Primers greatly improve formation of the desired products in multiplex PCR for seven and nine targets from two different genomic DNA sources. The development of a robust multiplex PCR assay typically requires an iterative design process to discover primer pairs that are both specific for the targets of interest and exhibit a low level of off-target amplicon formation. With CleanAmp™ Primers minimal design optimization is required.

The reduction or elimination of nonspecific amplifications achieved with the use of CleanAmp™ Primers makes them a powerful solution for improved one-step reverse-transcription (RT) PCR performance (Figure 10). By introducing CleanAmp™ Primers only the RT Primer can extend during the reverse transcription step of the protocol, resulting in specific amplification in a one tube, single step protocol.



## Reaching New Limits of Detection

CleanAmp™ Primers also offer an advantage over CleanAmp™ dNTPs in systems hindered by low template concentrations. A 10-100 fold increase in correct amplicon formation is seen when standard primers are replaced with CleanAmp™ Precision Primers (Figure 11). CleanAmp™ Primers greatly improve these systems by reducing or eliminating the off-target amplifications that compete with the desired amplicon. CleanAmp™ Primers have utility in many high-sensitivity downstream applications, such as single molecule detection.



## Product Details

CleanAmp™ Primers are available in two forms that differ in the rate of thermal activation. See table below for application based benefits. CleanAmp™ Primers are supplied cartridge purified. However, for those applications where PCR performance is of the utmost importance, we can RP-HPLC purify your CleanAmp™ Primers on a custom quote basis. Each primer undergoes quality control analysis by PAGE, MS and RP-HPLC to ensure high quality. CleanAmp™ Primers 15-40 bases in length are \$250/pair. We guarantee at least a 10 OD final yield from every synthesis.

CleanAmp™ Turbo Primers	CleanAmp™ Precision Primers
Fast cycling	Standard cycling
Multiplex PCR	One-step reverse-transcription PCR
Improves amplicon yield	Improves specificity and limit of detection
Reduces mis-priming/primer dimer formation	Greatest reduction in mis-priming/primer dimer formation

If you have oligonucleotide synthesis capabilities in-house, you may prefer making your own CleanAmp™ Primers. CleanAmp™ Amidites are available through our website, as well as through Glen Research at [www.glenres.com](http://www.glenres.com).

“Our work, in part, involves development of high sensitivity tests for mutations present in only a small fraction of cells under test. This requires high specificity also and it has proven to be the case that the TriLink CleanAmp™ Primers provided the specificity needed to capitalize on qPCR’s inherent high sensitivity. Using your primers we can routinely identify the presence of mutation fractions as low as 0.001%.”  
 - John Owen M.D., Professor of Hematology & Oncology at Wake Forest University

“We designed a quadruplex PCR assay to simultaneously amplify four herpesviruses, including herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein-Barr virus (EBV) and varicella zoster virus (VZV). We found that the use of CleanAmp™ Turbo Primers enhanced amplification efficacies in the quadruplex herpesvirus PCR assay where normal multiplex techniques previously failed.”  
 - Yi-Wei Tang, University of Vanderbilt

## TESTIMONIALS



TriLink BioTechnologies is an industry leader in manufacturing high quality oligonucleotides and nucleoside triphosphates at small and mid-scales.

TriLink operates highly compliant laboratories with Quality Assurance oversight and provides:

- Milligram to multi-gram synthesis
- Highly-modified oligonucleotides and nucleosides
- Oligonucleotide radiolabeling services
- Custom chemistry
- Contract research services
- Industry-leading technical support



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