Abstract
In a field where the demands for speed, sensitivity and specificity are high, PCR performance can be significantly improved by the use of Hot Start technologies. Hot Start dNTPs are a distinct approach that employs nucleoside triphosphates modified with a thermostable protecting group. This modification blocks low temperature primer extension and is released at higher temperatures to allow for more specific DNA polymerase incorporation. Hot Start dNTPs can be used with a variety of thermostable enzymes and can be applied to dNTP analogs such as 7-deaza-dGTP for improved GC-rich target amplification. The rate of deprotection allows for use in fast cycling PCR protocols. Furthermore, use of Hot Start dNTPs in multiplex assays improves sensitivity and specificity with standard and fast PCR protocols, as well as convenient one-step reverse transcription PCR (RT-PCR) protocols. As this technology continues to develop, its application to other molecular biology assays has great potential.

Figure 1: CleanAmp™ Hot Start dNTP Structure

Figure 2: CleanAmp™ Hot Start dNTP Activation

Figure 3: CleanAmp™ dNTPs Improve the Performance of DNA Polymerases

Figure 4: Comparable Performance to Other Hot Start Technologies

Figure 5: Broad Applications to Standard and Fast Thermal Cycling Protocols

Figure 6: Increased Yield and Specificity in Multiplex PCR

Figure 7: Efficient Fast Multiplex PCR

Figure 8: One-Step Reverse Transcription PCR (RT-PCR) Specificity is Improved by Hot Start Activation of the RT and PCR Steps

Figure 9: Quantification of RNA Expression Levels by Real-Time Hot Start One-Step RT-PCR

Figure 10: CleanAmp™ dNTPs Allow Simultaneous Detection of Multiple RNA Targets

Figure 11: PCR Amplification of GC-Rich Targets Improves Accuracy of Dideoxy Sequencing

Conclusion
• CleanAmp™ dNTPs improve PCR performance relative to standard dNTPs and provide comparable results to other Hot Start DNA technologies.
• CleanAmp™ dNTPs are compatible with a variety of enzymes and molecular biology assays.
• CleanAmp™ dNTPs can be employed in fast PCR cycling protocols to achieve high quality data in as little as 27 minutes.
• CleanAmp™ dNTPs enhance the specificity of multiplex PCR where amplification of at least 50 targets has been demonstrated.
• CleanAmp™ dNTPs improve one-step RT-PCR specificity by introducing Hot Start control to both the RT and PCR steps.


Contact: Natasha Paul, npaul@trilinkbiotech.com