Hot Start dNTPs - Novel Chemistries for Use in Advanced PCR Applications

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Abstract

PCR is a widely used scientific tool whose specificity can be increased by the use of Hot Start technologies. Although many Hot Start technologies exist, recently developed CleanAmp™ dNTPs are a distinct approach that employs modified nucleoside triphosphates with a thermolabile protecting group at the 3´-hydroxyl. The presence of the protecting group blocks low temperature primer extension, which can often be a significant problem in PCR. At higher temperatures, the protecting group is released to allow for incorporation by the DNA polymerase and more specific amplification of the intended target. These modified dNTPs provide comparable performance to other Hot Start technologies and can be used with thermostable DNA polymerases to turn a reaction into a Hot Start version. This thermolabile chemistry can be applied to dNTP analogs such as dUTP, which is used in UNG decontamination methods, and 7-deaza-dGTP, which is used to amplify difficult GC-rich targets. In addition, further studies have led to the development of 3´-protecting groups that deprotect more quickly than the current 3´-modification group, allowing these modified dNTPs to be used in fast PCR. CleanAmp™ dNTPs have provided a great improvement to traditional Hot Start PCR. With the evolving chemistry of CleanAmp™ dNTPs, the areas of application benefiting from the versatility and flexibility of this technology continue to grow.

Figure 1

Proposed activation mechanism of CleanAmp™ dNTPs

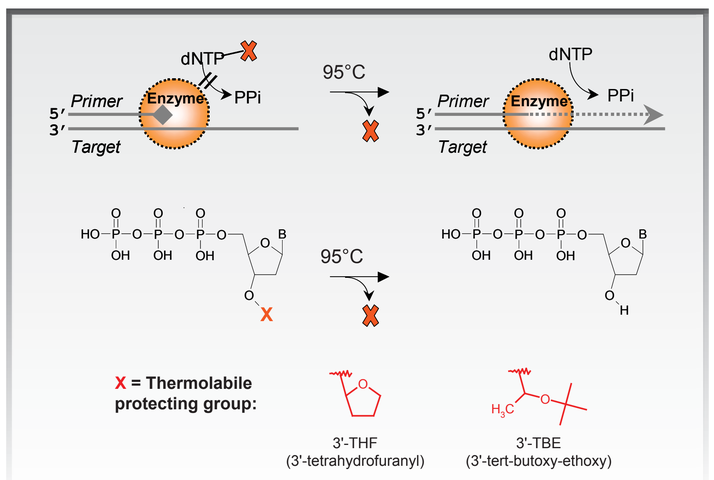
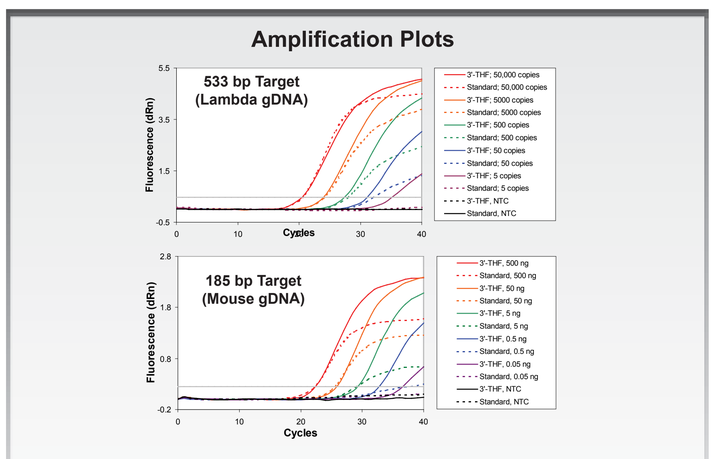


Figure 2

Evaluation of CleanAmp™ dNTPs in real-time PCR



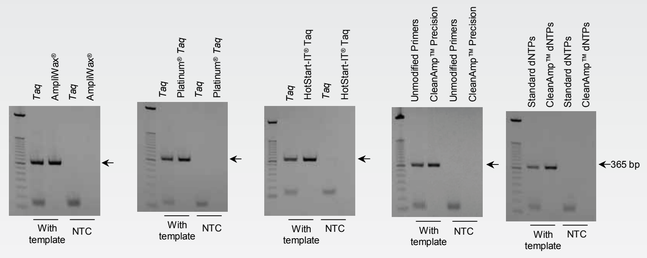
CleanAmp™ dNTPs improve the efficiency of PCR and increase the limit of detection in real-time PCR

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.2 µM), 0.2 mM dNTPs (standard or 3'-THF), 0-50,000 copies Lambda gDNA, 0-100 ng Mouse gDNA, TaqMan® probe (0.1 mM), ROX, 1.25 U *Taq* DNA polymerase, 25 µL.

Thermal cycling conditions: 533 bp target: 95°C (10 min); [95°C (40 sec), 57°C (30 sec), 72°C (1 min)] 40X.
185 bp target: 95°C (10 min); [95°C (30 sec), 60°C (30 sec), 72°C (30 sec)] 40X.

Figure 3

Comparison of CleanAmp™ dNTPs with other commonly used Hot Start technologies



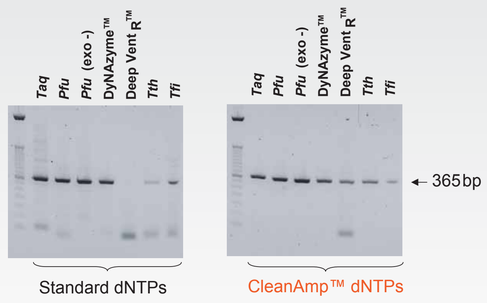
CleanAmp™ dNTPs provide similar performance to other Hot Start technologies

PCR conditions: 1X PCR buffer (specific for each DNA polymerase), Primers (0.2 µM), 0.2 mM dNTPs (standard or 3'-THF for reactions employing CleanAmp™) , 5 copies HIV-1 gDNA , 1.25 U DNA polymerase, 50 µL.

Thermal cycling conditions: AmpliWax® PCR Gem 50: Lower reaction mix: 80°C (5 min); 25°C (2 min), Add Upper reaction mix, then: 94°C (1 min); [94°C (45 sec), 56°C (30 sec), 72°C (1 min)] 35X; 72°C (7 min).
Platinum® *Taq* DNA polymerase: 94°C (2 min); [94°C (30 sec), 55°C (30 sec), 72°C (1 min)] 35X.
HotStart-IT® *Taq* DNA polymerase: 94°C (2 min); [94°C for (30 sec), 56°C (30 sec), 72°C (1 min)] 35X; 72°C (5 min).

Figure 4

Evaluation of CleanAmp™ dNTPs for use with thermostable DNA polymerases



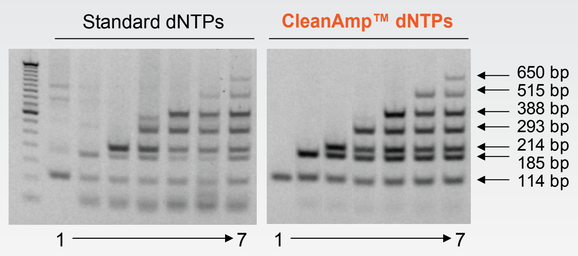
CleanAmp™ dNTPs improve the performance of a variety of thermostable DNA polymerases

PCR conditions: Primers (0.4 µM), 0.2 mM dNTPs (standard or 3'-THF), 5 copies HIV-1 gDNA, DNA polymerase (var. U), 50 µL.

Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2 min)] 35X; 72°C (7 min).

Figure 5

Comparison of standard and CleanAmp™ dNTPs in multiplex PCR



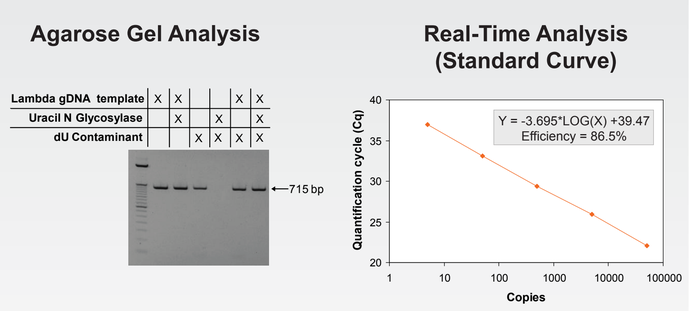
CleanAmp™ dNTPs can successfully amplify seven targets in multiplex PCR

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 70 mM KCl, 2.5 mM $MgCl_2$), Primers (0.2 µM), 0.4 mM dNTPs (standard or 3'-THF), 20 ng Mouse gDNA, 2.5 U *Taq* DNA polymerase, 25 µL

Thermal cycling conditions: 95°C (10 min); [95°C (30 sec), 60°C (30 sec), 72°C (30 sec and 1 min)] 35X; 72°C (5 min).

Figure 6

Development of CleanAmp™ dUTP for carryover decontamination PCR protocols



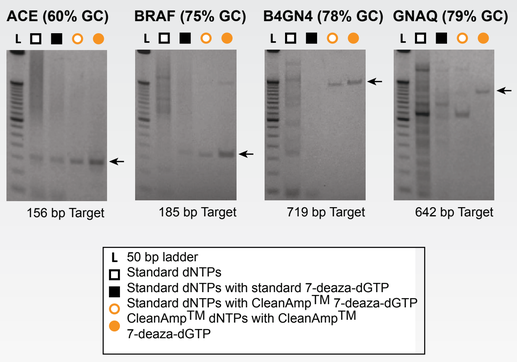
CleanAmp™ dUTP can be successfully used in UNG-mediated carryover decontamination schemes

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 40 mM KCl, 2.5 mM $MgCl_2$), 0.2 mM 3′-THF d(A,C,G)TP, 0.6 mM 3′-dUTP, Primers (0.2 µM), 1.25 U *Taq* DNA polymerase, 0-0.5 U UNG, 0 or 100,000 copies Lambda gDNA , +/- dU PCR contaminant, 25 µL.

Thermal cycling conditions: 25°C (5 min); 60°C (10 min); 95°C (10 min); [95°C (40 sec), 57°C (30 sec), 72°C (1 min)] 35X; 72°C (7 min).

Figure 7

Evaluation of CleanAmp™ 7-deaza-dGTP for amplification of targets with varied GC content



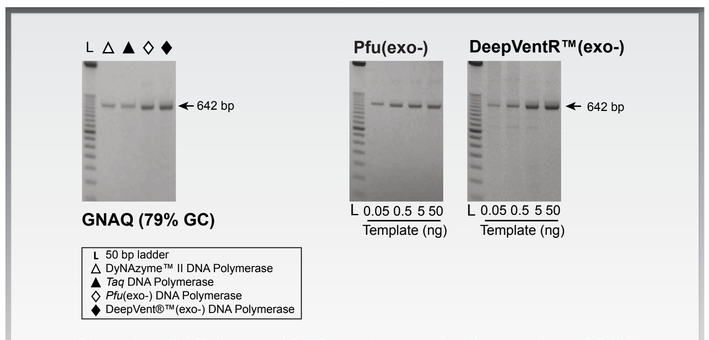
CleanAmp™ 7-deaza-dGTP can improve target specificity for GC-rich targets

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 40 mM KCl, 2.5 mM $MgCl_2$); Standard dNTPs = 0.2 mM d(A, C, G,T)TP; Reactions containing 7-deaza-dGTP contained 0.2 mM d(A, C, G,)TP (standard or 3'-THF); 0.05 mM dGTP (standard or 3'-THF) and 0.15 mM 7-deaza-dGTP (standard or 3'-THF); Primer conc.: 0.2 µM (ACE, B4GN4, GNAQ), 0.1 µM (BRAF); 1.25 U *Taq* DNA polymerase; 0.2 ng/µL Human gDNA; 25 µL.

Thermal cycling conditions: 95°C (10 min) [95°C (40 sec), X°C (1 sec), 72°C (1 min)] 35X, 72°C (7 min) (X= 66°C (ACE), 64°C (BRAF), 57°C (B4GN4), 66°C (GNAQ - subjected to 40 thermal cycles).

Figure 8

Evaluation of CleanAmp™ 7-deaza-dGTP for compatibility with DNA polymerases



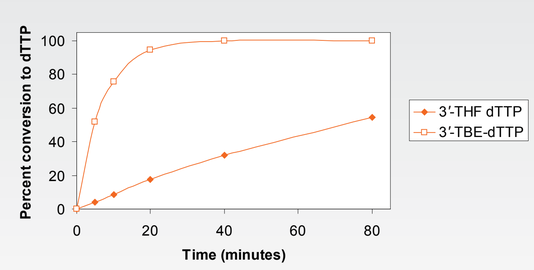
CleanAmp™ 7-deaza-dGTP can be used with a variety of DNA polymerases

PCR conditions: 1X PCR buffer (specific for DNA polymerase), 0.2 mM 3'-THF d(A, C, T)TP, 0.05 mM 3'-THF dGTP, 0.15 mM 3'-THF 7-deaza-dGTP; Primer conc: 0.2 µM; Human gDNA 0-50 ng; DNA polymerases (and final Mg^{2+} concentration): DyNAzyme™ II (1.5 mM $MgCl_2$), *Taq* DNA polymerase (2.5 mM $MgCl_2$), *Pfu*(exo-) (2 mM $MgSO_4$), DeepVent®™(exo-) (2 mM $MgSO_4$); 25 µL.

Thermal cycling conditions: 95°C (10 min) [95°C (40 sec), 66°C (1 sec), 72°C (1 min)] 40X, 72°C (7 min).

Figure 9

Assessment of the deprotection kinetics of faster CleanAmp™ dNTP analogs

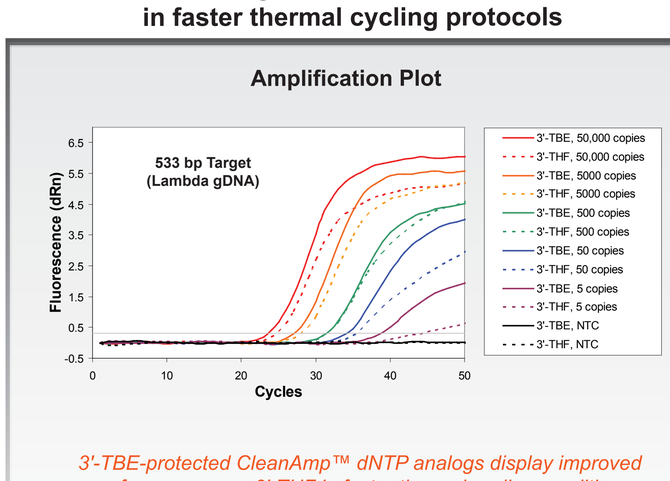


3'-TBE-dTTP deprotects more than10-fold faster than 3'-THF-dTTP in PCR buffer at elevated temperatures

Deprotection conditions: 1X PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris (pH 8.4 at 25°C)); 0.25 mM 3′-THF-dTTP or 3′-TBE-dTTP, 95°C incubation.

Figure 10

Evaluation of second generation CleanAmp™ dNTP analogs in faster thermal cycling protocols



3'-TBE-protected CleanAmp™ dNTP analogs display improved performance over 3'-THF in faster thermal cycling conditions

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 40 mM KCl, 4 mM $MgCl_2$), 0.4 mM 3′-TBE-dNTPs or 3′-THF-dNTPs, Primer conc: 0.5 µM, 5 U Taq DNA polymerase, 0-50,000 copies Lambda gDNA, 25 µL.

Thermal cycling conditions: [95°C (10 sec), 65°C (30 sec)] 50X.

Conclusion

1) CleanAmp™ dNTPs improve PCR performance relative to standard dNTPs for various targets of different lengths.

2) CleanAmp™ dNTPs provide comparable results to other Hot Start DNA polymerases.

3) CleanAmp™ dNTPs enrich PCR specificity when used with a variety of thermostable DNA polymerases.

4) CleanAmp™ dNTPs enhance reaction efficiency in multiplex PCR, allowing amplification of up to seven targets.

5) CleanAmp™ dUTP can be incorporated in UNG decontamination schemes.

6) CleanAmp™ 7-deaza-dGTP improves the amplification of GC-rich targets and can be used with a variety of thermostable DNA polymerases.

7) Second generation CleanAmp™ dNTPs show promise for use in faster PCR cycling protocols.

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