Hot Start dNTPs - A Novel Tool for Controlled Nucleotide Incorporation in PCR

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Abstract

PCR is a widely used scientific tool employed by a variety of applications. Various Hot Start technologies have already been developed using modified PCR components to increase specificity of a reaction. Recently developed CleanAmpTM dNTPs are modified nucleoside triphosphates with a thermolabile 3'-tetrahydrofuranyl protecting group that is released at higher temperatures. These modified dNTPs prevent low temperature primer extension, which can often be a significant problem in PCR. At higher temperatures, the modified dNTPs are deprotected, to allow for incorporation by the DNA polymerase and more specific amplification of the intended target. The use of CleanAmpTM dNTPs provides comparable performance to other Hot Start technologies and shows promise to provide a synergistic effect when used in conjunction with other Hot Start methods. This modified dNTP technology also has the ability to use any DNA polymerase in a Hot Startsystem, which can be very cost effective. Although the utility of CleanAmpTM dNTPs in traditional Hot Start PCR has been previously demonstrated, they can also be used in more advanced PCR applications that require temperature-controlled nucleotide incorporation. In these advanced applications, the CleanAmp TM dNTP modification blocks nucleotide incorporation during initial, lower temperature reactions, allowing for delayed nucleotide activation in a reaction. In summary, CleanAmpTM dNTPs have the potential to provide great versatility and flexibility in a vast number of applications including traditional Hot Start PCR.

Figure 1

Proposed activation mechanism of CleanAmp™ dNTPs

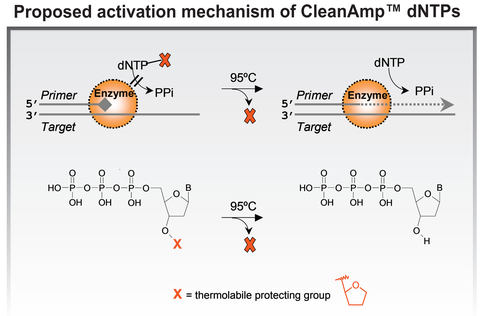
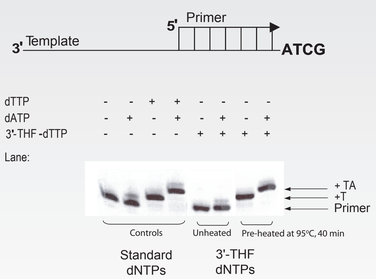


Figure 2

Primer Extension with Klenow fragment of DNA polymerase I using CleanAmp™ dTTP

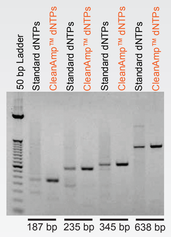


3'-THF dTTP is not incorporated by DNA polymerase prior to a Hot Start activation step

Reaction Conditions: 12.5 µM primer, 20 µM template, 1x reaction buffer (10 mM Tris-HCl (pH 7.9), 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT), 0.17 mM dNTPs, and 0.5 U Klenow (exo-) DNA polymerase, 50 µL.
The reaction mixture was incubated for 40 min. at 25ºC.

Figure 3

Comparison of standard dNTPs and CleanAmp™ dNTPs in targets of varying lengths



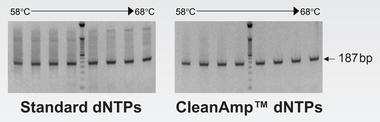
CleanAmp™ dNTPs demonstrate greatest amplicon yield and off-target reduction relative to unmodified dNTPs across targets of varying lengths

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, 1.25 U *Taq* DNA polymerase, 50 µL. Template: 1 ng Human gDNA.

Thermal cycling conditions: 95ºC (10 min); [95ºC (15 sec), 63ºC (30 sec), 72ºC (60 sec)] 35X; 72ºC (5 min).

Figure 4

Evaluation of standard dNTPs and CleanAmp™ dNTPs at different annealing temperatures



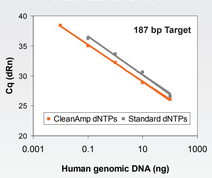
CleanAmp™ dNTPs reduce or eliminate off-target amplicons formation across a range of annealing temperatures

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, 1.25 U *Taq* DNA polymerase, 50 µL. Template: 1 ng Human gDNA.

Thermal cycling conditions: 95ºC (10 min); [95ºC (15 sec), 63ºC (30 sec), 72ºC (60 sec)] 35X; 72ºC (5 min).

Figure 5

Real-time PCR performance using CleanAmp™ dNTPs



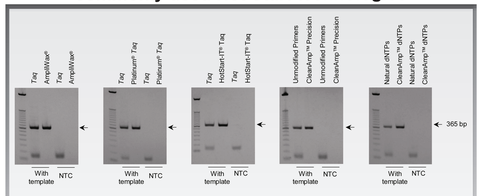
CleanAmp™ dNTPs improve the limit of detection in Real-time PCR without the need for Hot Start DNA polymerase

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 40 mM KCl, 2.5 mM $MgCl_2$), Primer conc: (0.2µM), 0.2 mM dNTPs, 0.01-100 ng Human gDNA, 1.25 U *Taq* DNA polymerase, 50 µL.

Thermal cycling conditions: 95ºC (10 min); [95ºC (15 sec), 63ºC (30 sec), 72ºC (1 min)] 35X, 72ºC (5 min).

Figure 6

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

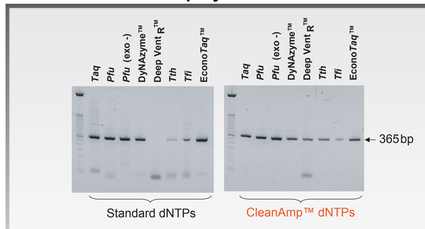


CleanAmp™ dNTPs provide similar performance to other Hot Start technologies

PCR conditions: 1X PCR buffer (specific for each DNA polymerase), Primers (0.2 µM), 0.2 mM dNTPs, 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).
Taq, CleanAmp™ primers and CleanAmp™ dNTPs: 94°C (10 min); [94°C @ 40 sec., 57°C @ 30 sec., 72°C @ 1 min] 35X;

Figure 7

Compatibility of CleanAmp™ dNTPs with DNA polymerases



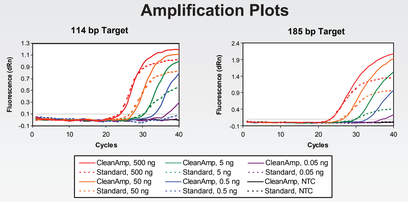
CleanAmp™ dNTPs can improve the performance of a variety of thermostable DNA polymerases

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

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

Figure 8

Multiplex qPCR performance using CleanAmp™ dNTPs



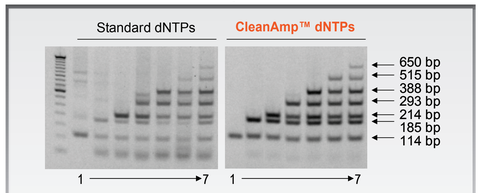
CleanAmp™ dNTPs improve the efficiency of PCR and increase limit of detection in multiplex PCR

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 40 mM KCl, 2.5 mM $MgCl_2$), 0.2 mM dNTPs, 0.05-500ng Mouse gDNA, 2.5 U *Taq* DNA polymerase, Primer conc: (0.2µM), 50 µL.

Thermal cycling conditions: 95ºC (10 min); [95ºC (15 sec), 60ºC (30 sec), 72ºC (1 min)] 35X; 72ºC (5 min).

Figure 9

Comparison of Standard dNTPs and CleanAmp™ dNTPs in multiplex PCR



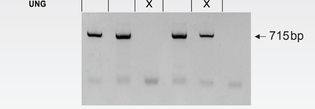
CleanAmp™ dNTPs are able to successfully amplify seven targets in multiplex PCR

PCR conditions: 1X PCR buffer (20 Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.2 µM), 0.4 dNTPs and THF dNTPs, 20 ng Mouse gDNA, 2.5 U *Taq* DNA polymerase, 25 µL.

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

Figure 10

Use of CleanAmp™ dUTP with UNG decontamination protocols



CleanAmp™ dUTP is compatible with UNG decontamination protocols

PCR conditions: 1X PCR buffer (20 Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.2 µM), 0.2mM dNTPs and THF dNTPs, 100,000 copies Lambda gDNA, 1:100 CleanAmp™ dUTP Contaminant, 1.25 U *Taq* DNA polymerase, 25 µL.

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

Conclusion

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

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

3) CleanAmp™ dNTPs improve the limit of detection 10-fold relative to traditional PCR protocols.

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

5) CleanAmp™ dNTPs enhance reaction efficiency in multiplex PCR and can amplify up to seven targets.

6) CleanAmp™ dUTPs are able to be incorporated in UNG decomination schemes.

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