TriLink BIOTECHNOLOGIES

CleanAmp™ 7-deaza-dGTP
Catalog # N-9515

When DNA targets high in GC content are amplified, 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 noteable method for overcoming this problem. TriLink has 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. The CleanAmp™ dNTP Hot Start technology employs a thermolabile protecting group at the 3' hydroxyl. The presence of this modification blocks DNA polymerase nucleotide incorporation until the nucleotide protecting group is removed using a heat activation step. When standard cycling protocols are employed, a 0-10 minute initial denaturation step at 95°C allows for robust amplification. CleanAmp™ 7-deaza-dGTP is available on its own for amplification of GC-rich targets or as the CleanAmp™ 7-deaza-dGTP Mix, recommended for more challenging targets with higher GC content.

N-9515-2 (2 µmoles)
N-9515-10 (10 µmoles)

50 mM solution
Sodium Salt form

Store at -20°C or below

QC Analysis

Functional Assay; Pass
Components tested in standardized PCR assay

AX-HPLC; Pass
31P NMR; Pass
RP-HPLC; Pass
Mass Spec; Pass

Product released by Quality Assurance

Handling

CleanAmp™ dNTPs are characteristically very similar to natural dNTPs and are stable in aqueous buffer at pH 8-10.5 for at least one year at -20° C. When stored incorrectly, the major point for degradation of both natural and CleanAmp™ dNTPs is the triphosphate chain. Much like natural dNTPs, extra care should be taken not to expose CleanAmp™ dNTPs to prolonged temperatures above 20°C. Exposure to ambient temperatures during shipping does not adversely affect product performance. We do not recommend exposure of the CleanAmp™ dNTP stock solution to more than 24 TOTAL hours at room temperature or subjecting CleanAmp™ dNTPs to more than 20 freeze-thaw cycles. If greater than ten freeze-thaw cycles are anticipated, distribute your stock solution accordingly into smaller aliquots.

Use

CleanAmp™ 7-deaza-dGTP can be directly substituted for the natural nucleotides and has been evaluated on targets up to 1,200 bp long, containing up to 80% GC-content. See reverse for standard thermal cycling protocol and dNTP mixture preparation instructions. Should the CleanAmp™ dNTP concentration or units of DNA polymerase be increased, the $MgCl_2$ concentration should also be increased. Our data shows good PCR performance over a primer concentration range of 0.05 µM to 0.5 µM, finding 0.2 µM to work well in most cases.

Standard Thermal Cycling: 25 µL Endpoint PCR

1. For all components except CleanAmp™ dNTPs and DNA polymerase, thaw the reaction components, vortex to mix, centrifuge briefly and store on ice.
2. Prepare CleanAmp™ dNTPs:
   a. Thaw at room temperature or on ice.
   b. Vortex and pulse centrifuge to thoroughly mix.
   c. Remove an aliquot of the stock solution and dilute with water or buffer (pH 8-10.5) and prepare mixture according to Table 1 below.

Table 1

Component	Stock Concentration[2]	Desired Concentration[2]	Volume
dATP[1]	100 mM	10 mM	2 µL
dCTP[1]	100 mM	10 mM	2 µL
dGTP[1]	100 mM	2.5 mM	0.5 µL
CleanAmp™ 7-deaza-dGTP	50 mM	7.5 mM	3 µL
dTTP[1]	100 mM	10 mM	2 µL
Sterile Deionized Water	-	-	10.2 µL
Total Volume	-	-	20 µL

4. Prepare a mastermix containing all components except for the DNA template sample. Add each of the components as shown in Table 2 (multiply amounts by the number of reactions needed) in a centrifuge tube, on ice.

Table 2

Component	Final Concentration (in a 25 µL reaction)	Volume for 1 reaction	Volume for 10 reactions
Forward/Reverse Primer	50-500 nM	Variable	Variable
Sterile de-ionized water	Up to 25 µL	Up to 25 µL	Up to 250 µL
$MgCl_2$ (50 mM)[3]	2.5 mM	1.25 µL	12.5 µL
PCR buffer 10X[4]	1X	2.5 µL	25 µL
CleanAmp™ 7-deaza-dGTP Mix (10mM)[5]	0.2 mM	0.5 µL	5 µL
Taq 5 U/µL	0.05 U/µL	0.25 µL	2.5 µL
Template	Variable	5 µL	50 µL
Total Volume (µL)	**25 µL**	**25 µL**	**250 µL**

6. Mix the mastermix gently to protect the enzyme, by pipetting up and down. (do not vortex.) Pulse spin if necessary.
7. Aliquot 20 µL of mastermix into each thin-walled PCR tube.
8. To each 20 µL aliquot of mastermix, add 5 µL of the appropriate template DNA for a final reaction volume of 25 µL.
9. Pulse spin PCR tubes to remove bubbles and collect reaction solution at bottom of tube.
10. Place the tubes into a thermal cycler with a heated lid and perform the appropriate cycling conditions for GC-rich amplification:
    95°C for 10 min
    [95°C for 40 sec; X°C[7] for 1 sec[8]; 72°C for 0.5-2 min]
    35-40 cycles 72°C for 10 min
    For systems > 70% GC-rich 40 cycles are suggested to improve yield.
11. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

[1] Standard or CleanAmp™ dNTPs can be used.
[2] Typical stock concentrations shown. Adjust volumes accordingly, as needed.
[3] For protocols utilizing alternate DNA polymerases, the MgCl2 concentration can be optimized between 2.5 and 4.0 mM.
[4] 10X PCR buffer for Invitrogen's Taq DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25°C; 500 mM KCl)
[5] If CleanAmpTM 7-deaza-dGTP (N-9515) is used, a 1:3 ratio of dGTP (standard or CleanAmpTM) to CleanAmpTM 7-deaza-dGTP is recommended.
[6] The annealing temperature should be chosen for optimal PCR performance. Most primer design software recommends an annealing temperature. The annealing temperature can also be optimized experimentally by using a thermal cycler with gradient functionality or by performing sequential experiments in which the annealing temperature is varied.
[7] A 1 sec annealing time is important for specificity.

Troubleshooting

- Should excess banding be seen; try using less DNA polymerase.
- The annealing temperature should be chosen for optimal PCR performance.
- The $MgCl_2$ concentration can be optimized between 2.5 and 4.0 mM.
- 10X PCR buffer for Invitrogen's *Taq* DNA polymerase = 200 mM Tris-HCl, pH 8.4 at 25°C; 500 mM KCl.
- For improved performance, the CleanAmp™ dNTP concentration can be increased. For every additional 0.2 mM concentration of CleanAmp™ dNTPs, add at least an additional 1.0 mM of $MgCl_2$.
- If amplicon yield is low: try substituting *Pfu* (exo-) or Deep $Vent_R$™ exo- DNA polymerases for *Taq* DNA polymerase or add additional thermal cycles.
- Use CleanAmp™ 7-deaza-dGTP (N-9515) with standard dNTPs for targets with moderate GC content (~60%). Use CleanAmp™ 7-deaza-dGTP Mix (N-9504) for targets with GC content greater than 60%.
- Titrate template concentrations for better specificity. 2 ng/µl template concentration works well for most targets.