# CleanAmp™ 7-deaza-dGTP Mix
Catalog # N-9504

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-9504-2 (2 µmoles of each component)
N-9504-10 (10 µmoles of each component)

Sodium Salt form

Store at -20°C or below

| Catalog Number | Component | Concentration |
|---|---|---|
| N-9520 | CleanAmp™ dATP | 10 mM |
| N-9521 | CleanAmp™ dCTP | 10 mM |
| N-9522 | CleanAmp™ dGTP* | 2.5 mM |
| N-9515 | CleanAmp™ 7-deaza-dGTP* | 7.5 mM |
| N-9523 | CleanAmp™ dTTP | 10 mM |

*1:3 ratio of dGTP:7-deaza-dGTP

## QC Analysis

Functional Assay; Pass
Components tested in standardized PCR assay

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

*Components analyzed separately

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 Mix can be directly substituted for the natural nucleotides and has been evaluated on targets up to 1200 bp long, containing up to 80% GC-content. For standard thermal cycling protocols, we recommend 2.5 mM MgCl2, 200 µM CleanAmp™ 7-deaza-dGTP Mix and 1.25 units of Taq DNA polymerase. Should the CleanAmp™ dNTP concentration or units of DNA polymerase be increased, the MgCl2 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. If necessary, remove an aliquot of the stock solution and dilute with water or buffer (pH 8-10.5) to desired working concentration.
3. Prepare a mastermix containing all components except for the DNA template sample. Add each of the components as shown in Table 1 (multiply amounts by the number of reactions needed) in a centrifuge tube, on ice.

Table 1

| 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)[1] | 2.5 mM | 1.25 µL | 12.5 µL |
| PCR buffer 10X[2] | 1X | 2.5 µL | 25 µL |
| CleanAmp™ 7-deaza-dGTP Mix (10mM)[3] | 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** |

4. Mix the mastermix gently to protect the enzyme, by pipetting up and down. (Do not Vortex.) Pulse spin if necessary.
5. Aliquot 20 µL of mastermix into each thin-walled PCR tube.
6. To each 20 µL aliquot of mastermix, add 5 µL of the appropriate template DNA for a final reaction volume of 25 µL.
7. Pulse spin PCR tubes to remove bubbles and collect reaction solution at bottom of tube.
8. 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[4] for 1 sec[5]; 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.
9. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

[10] For protocols utilizing alternate DNA polymerases, the $MgCl_2$ concentration can be optimized between 2.5 and 4.0 mM.
[11] 10X PCR buffer for Invitrogen's Taq DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25°C; 500 mM KCl)
[12] If CleanAmp™ 7-deaza-dGTP (N-9515) is used, a 1:3 ratio of dGTP (standard or CleanAmp™) to CleanAmp™ 7-deaza-dGTP is recommended.
[1] 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.
[2] A 1 sec annealing time is important for specificity.

## Real-Time PCR

GC amplification protocols should be compatible with real-time reagents with the following recommendations. Additional real-time components for SYTO® 9 detection:
• SYTO® 9 Green Fluorescent Nuc. Acid Stain - 5 mM (Invitrogen)
• Passive reference ROX dye - 1 mM (Agilent)

Adaptation to real-time PCR: The reaction setup should be identical to the setup described above, except for the additions of the real-time components. Reactions should be incubated in a thermal cycler capable of real-time detection, where fluorescence data is collected at the completion of the annealing step of each cycle. Please contact the real-time instrument manufacturer for specific details on your setup. For SYTO® 9-based detection, 30 or 300 nM passive ROX reference dye and 2 µM SYTO® 9 Nucleic Acid Stain should be included in the reaction.

Note: The dilution of passive ROX reference dye can be kept at 4°C for approximately one month. For the passive ROX reference dye and SYTO® 9, keep tubes protected from light at all times (i.e. wrap tubes with aluminum foil).

Note: Reactions should be set up in the appropriate optical tubes recommended by the instrument manufacturer. Wear powder-free gloves when performing all real-time setups. Optical tubes and caps should be wiped with Kimwipe® to remove any residue that may interfere with optical reading.

## 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.