CleanAmp™ dNTP Mix

Catalog # N-9506

N-9506-2 (2 µmoles of each component)
N-9506-10 (10 µmoles of each component)
N-9506-BK (Bulk amount 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	10 mM
N-9523	CleanAmp™ dTTP	10 mM

QC Analysis

Functional Assay; Pass
Components tested in standardized PCR assay

AX-HPLC*; Pass
RP-HPLC*; Pass
^{31}P NMR*; Pass
Mass Spec*; Pass
*Components analyzed separately

Product released by Quality Assurance

Product Overview

CleanAmp™ dNTPs help to control mis-priming and primer dimer formation by blocking DNA polymerase nucleotide incorporation until elevated temperatures are achieved. Like other Hot Start approaches, these modified nucleoside triphosphates are activated by the elevated temperatures of PCR thermal cycling. Replacement of the essential DNA polymerase substrate, the dNTPs, with the recommended concentration of CleanAmp™ dNTPs allows for use in existing PCR protocols. CleanAmp™ dNTPs offer excellent results in a number of PCR-based applications for a fraction of the cost of other Hot Start solutions.

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™ dNTPs were designed to be used as a replacement for natural nucleotides in reactions using standard thermophilic DNA polymerases such as *Taq*. Table 1 lists the enzymes qualified for use with CleanAmp™ dNTPs using basic PCR primer template systems, which amplify genomic DNA or plasmid targets. For standard thermal cycling protocols, we recommend 2.5 mM $MgCl_2$, 400 µM CleanAmp™ dNTPs and 1.25 units of *Taq* DNA polymerase. 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.

Table 1

DNA Polymerase	Vendor	pH/Reaction	Units/µL
Taq	Invitrogen	8.4	5.0
Taq	New England Biolabs	8.3	5.0
Taq	USB	8.6	5.0
Pfu	Stratagene	8.8	2.5
Pfu (exo-)	Stratagene	8.8	2.5
DyNAzyme™	Finnzymes	8.8	2.0
Deep Vent$_R$™ (exo-)	New England Biolabs	8.8	2.0
Tth	USB	8.6	5.0
Tfi	Invitrogen	8.4	5.0

See Reverse for Protocols and Troubleshooting

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 2 (multiply amounts by the number of reactions needed) in a centrifuge tube, on ice.
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 standard thermal cycling:
   95°C for 0-5 min
   [95°C for 10 sec; 48-60°C[1] for 1-30 sec; 72°C for 0.5-2 min] 30-40 cycles
   72°C for 10 min
9. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

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 20 µL	Up to 20 µL	Up to 200 µL
$MgCl_2$ (50 mM)[2]	2.5 mM	1.25 µL	12.5 µL
PCR buffer 10X[3]	1X	2.5 µL	25 µL
CleanAmp™ dNTP solution[4,5]	0.4 mM	1 µL	10 µL
Taq DNA polymerase (5µ/µL)	0.05 units/µL	0.25 µL	2.5 µL
Total Volume (µL)	**20 µL**	**20 µL**	**200 µL**

[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] For protocols utilizing alternate DNA polymerases, the $MgCl_2$ concentration can be optimized between 2.5 and 4.0 mM.
[3] 10X PCR buffer for Invitrogen's *Taq* DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25ºC; 500 mM KCl)
When using CleanAmp™ dNTP Mix (Cat # N-9506), use the 10 mM stock solution as is. To test the effect of CleanAmp™ dNTPs on an individual basis, prepare a solution in which one or more of the standard dNTPs (dA, dC, dG, or dT) are replaced with the corresponding CleanAmp™ dNTPs.
[5] 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 $MgCl_2$.

Real-Time

The standard cycling protocol can be adapted for real-time experiments with the following alterations:

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 SYBR® Green I-based detection, 30 or 300 nM passive ROX reference dye (1 mM, Agilent) and 0.15X SYBR® Green I Nucleic Acid Stain (10,000X, Invitrogen) should be included in the reaction.
- For SYTO® 9-based detection, 30 or 300 nM passive ROX reference dye (1 mM, Agilent) and 2 µM SYTO® 9 Nucleic Acid Stain (5 mM, Invitrogen) should be included in the reaction.
- For hydrolysis probe-based detection, 30 or 300 nM passive ROX reference dye (1 mM, Agilent) and 50-20 nM hydrolysis probe should be included. The optimal hydrolysis probe concentration should be determined by performing serial dilutions and identifying the concentration that provides the earliest Cq and maximal fluorescence intensity.

Note: The dilution of passive ROX reference dye can be kept at 4°C for approximately one month. For the passive ROX reference dye, hydrolysis probe, SYBR® Green 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

Probable Cause	Suggestion(s)
No amplification product or low amplicon yield	
Insufficient activation of CleanAmp™ dNTPs during thermal cycling	Increase the concentration of CleanAmp™ dNTPs to up to 0.8 mM, adding $MgCl_2$ to up to 4.0 mM.
	Optimize the duration of the initial denaturation time to up to 10 minutes.
Thermal cycling protocol is not optimized	Increase extension time. Generally extension times should be 1-2 minutes per kb of target.
	Increase the number of thermal cycles in 5 cycle increments.
	Optimize annealing temperature.
Problem with reagents or reaction conditions	Prepare fresh reagents, including reaction buffer and dNTPs.
	Verify that template is good in quality and of sufficient quantity.
	Verify primer design to ensure adequate complementarity to the DNA target.
	Optimize the $MgCl_2$ concentration (2.5 to 4.0 mM final conc.).
Non-specific product formation	
Excessive off-target primer extension	Titrate the concentration of the primers or template DNA.
	Reduce the amount of DNA polymerase.
Primer dimer formation	Reduce initial denaturation and denaturation times: 95°C for 0-5 min, [95°C for 10-20 sec, 48-60°C for 1-15 sec, 72°C for 0.5-2 min] 30-40 cycles, 72°C for 10 min Note A zero initial denaturation time in primer/template systems prone to primer dimer formation may cause a slight delay in Cq
Mis-priming	Omit initial denaturation time and shorten annealing time: [95°C for 30 sec, 48-60°C for 1 sec, 72°C for 0.5-2 min] 30-40 cycles 72°C for 10 min

Contact

TriLink BioTechnologies, Inc.
Info@trilinkbiotech.com
9955 Mesa Rim Road | San Diego, CA 92121
P(858) 546.0004 :: (800) 863.6801 | F(858) 546.0020