



CleanAmp™ dNTPs

The Next Generation in Hot Start PCR

Product Manual



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Materials Provided

CleanAmp™ dNTP Mix: dATP, dCTP, dGTP and dTTP

Catalog #	Quantity	Volume*
N-9501-2	2 μ mole each (4 x 2 μ moles)	200 μ l
N-9501-10	10 μ mole each (4 x 10 μ moles)	1000 μ l

* Each nucleotide is present at 10 mM concentration

Storage Conditions

Upon receipt, store at -20°C or lower. Do not expose the stock solution to more than 10 total hours at room temperature.

Product Introduction

CleanAmp™ dNTPs are the newest addition to TriLink's CleanAmp™ line of PCR enhancing products. Our CleanAmp™ Products offer a complete chemical solution to Hot Start activation through two widely ignored components of the PCR reagent mix: the primers and the dNTPs.

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 solutions, these modified nucleoside triphosphates are activated by the elevated temperatures of PCR thermal cycling. CleanAmp™ dNTPs offer precise control at the start of PCR thermal cycling by blocking DNA polymerase nucleotide incorporation until activated, thereby vastly improving PCR specificity.

CleanAmp™ dNTPs offer a general Hot Start solution for PCR. 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 are compatible

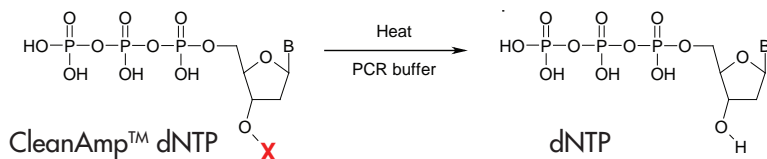
with existing primer sets and with a number of thermostable DNA polymerases. In addition, CleanAmp™ dNTPs can be used in combination with other Hot Start solutions for greater benefit. CleanAmp™ dNTPs offer excellent results in a number of PCR-based applications for a fraction of the cost of other Hot Start solutions.

Benefits

- Inexpensive compared to other Hot Start technologies
- Greatly reduces and often eliminates off-target amplicon formation, such as primer dimers
- Improves PCR specificity which leads to a much higher yield of the desired amplicon
- Validated for use with many PCR systems, by the replacement of the natural dNTPs with CleanAmp™ dNTPs
- Water soluble, with comparable stability to natural nucleotides under normal storage conditions
- Compatible with both Hot Start and non-Hot Start DNA polymerases that employ different buffer compositions, pH 7.5 up to pH 9, at 25°C

Details

CleanAmp™ dNTPs are modified with a thermolabile protecting group (X) at the 3' terminus. 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 2-10 minute initial denaturation step at 95°C allows for robust amplification. For faster thermal cycling protocols, an initial denaturation is not required. In many cases, all that is needed to successfully utilize CleanAmp™ dNTPs in a PCR reaction is to replace the natural nucleotides with CleanAmp™ dNTPs. See pages 7-11 for the concentration of CleanAmp™ dNTPs we recommend for your application.



X = thermolabile protecting group

Although we recommend using the CleanAmp™ dNTP Mix, which contains the modified nucleoside triphosphates of dA, dC, dG and dT, we have found that sometimes the replacement of just one or two natural nucleotides with CleanAmp™ dNTPs is enough to have the desired effect. CleanAmp™ dNTPs are available individually. We have recommended optimal conditions for commonly-used applications, which are described on pages 7-11, however good results can be obtained with a wide variety of conditions.

Handling Prior to Use

In our ongoing stability study, CleanAmp™ dNTPs are remarkably stable in aqueous buffer at pH 8-10.5 for at least six months. At elevated temperature (45°C), the stability of the 5' triphosphate moiety of CleanAmp™ dNTPs is similar to that of standard, unmodified dNTPs. Furthermore, provided they are stored in an aqueous buffer within the appropriate pH range, CleanAmp™ dNTPs do not need special handling. Much like natural dNTPs, extra care should be taken not to expose CleanAmp™ dNTPs to prolonged temperatures above 4°C. Although reaction mixtures that contain the CleanAmp™ dNTPs are stable at room temperature, we do not recommend exposure of the CleanAmp™ dNTP Mix stock solution to more than 10 TOTAL hours at room temperature. To avoid prolonged exposure of the CleanAmp™ dNTP Mix stock solution at room temperature, store stock nucleotide solutions in the freezer (-20°C) and store only one week's worth of material in the refrigerator. We recommend not subjecting CleanAmp™ dNTPs

to more than ten freeze-thaw cycles and distributing your stock solution into smaller aliquots that are sufficient for one week of work.

Handling Guidelines

1. The CleanAmp™ dNTP mix is shipped as a concentrated 10 mM solution of dATP, dCTP, dGTP and dTTP. They can be diluted into a PCR buffer solution (pH range of most PCR buffers = 8 to 9) and refrozen at -20°C in smaller aliquots to ensure stability for at least six months. CleanAmp™ dNTPs are very stable in the stock solution in which they are delivered.
2. CleanAmp™ dNTPs can be stored for up to one week at 4°C as the CleanAmp™ dNTP Mix or diluted in PCR buffer (pH 8 to 9).
3. We do not recommend storing CleanAmp™ dNTPs at room temperature. CleanAmp™ dNTPs should be thawed at room temperature or on ice, mixed by vortexing and pulse centrifugation and stored on ice during PCR set up or aliquoting manipulations. The CleanAmp™ dNTP stock solution should not be exposed to more than 10 TOTAL hours at room temperature. Do NOT thaw CleanAmp™ dNTPs by heating.
4. Although we do not have extensive experience with the stability of CleanAmp™ dNTPs in complete PCR master mixes, our data suggests that the CleanAmp™ dNTPs are stable in the presence of enzymes and other reagents commonly used in PCR reactions.
5. Although CleanAmp™ dNTPs improve PCR performance when used with standard primers and a non-Hot Start DNA polymerase, we have found some instances where CleanAmp™ dNTPs provide a further benefit in combination with other Hot Start reagents. One combination that shows significant benefit is the use of CleanAmp™ dNTPs in conjunction with AmpliTaq Gold® DNA polymerase (Applied Biosystems) when an amplification is prone to mis-priming.

Product Use

CleanAmp™ dNTPs were designed to be used as a replacement for natural nucleotides in reactions using standard thermophilic DNA polymerases such as *Taq* and *Pfu*. Table 1 below lists the enzymes qualified for use with CleanAmp™ dNTPs using basic PCR primer template systems, which amplified genomic DNA or plasmid targets. We are continually testing more enzymes; contact us to see if we have tested the one of interest to you. We have tested enzymes that employ reaction buffers which range in pH from 8 to 9 with good results. This wide range of compatible reaction buffers allows for a great deal of flexibility in DNA polymerase choice in PCR design.

Table 1:

DNA Polymerase	Vendor	pH/Reaction	Units/ μ L
<i>Taq</i>	Invitrogen	8.4	5.0
<i>Pfu</i>	Stratagene	8.8	2.5
<i>Pfu</i> (exo-)	Stratagene	8.8	2.5
<i>DyNAzyme</i> ™	Finnzymes	8.8	2.0
<i>Deep Vent</i> _R ™	NE Biolabs	8.8	2.0
<i>Tth</i>	USB	8.6	5.0
<i>Tfi</i>	Invitrogen	8.4	5.0
<i>EconoTaq</i> ™	Lucigen	9.0	5.0

Specific Reaction Conditions for Standard Cycling Protocols:

Taq DNA polymerase

- Reaction Buffer: 10X PCR buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl) plus $MgCl_2$ to a 2.5 mM final concentration.
- DNA polymerase Stock: *Taq* DNA pol (5 U/ μ L); amount per reaction = 1.25 U (0.25 μ L)

Pfu (exo+) and (exo-) DNA polymerase

- Reaction Buffer: 10X Cloned *Pfu* buffer
- DNA polymerase Stock:
 - Cloned *Pfu* (exo+) DNA pol (2.5 U/ μ L); amount per reaction = 2.5 U (1.0 μ L)
 - Cloned *Pfu* (exo-) DNA pol (2.5 U/ μ L); amount per reaction = 2.5 U (1.0 μ L)

DyNAzyme™ II DNA polymerase

- Reaction Buffer: 10X Optimized DyNAzyme™ Buffer
- DNA polymerase Stock: DyNAzyme™ II DNA pol (2 U/ μ L); amount per reaction = 1 U (0.5 μ L)

Deep Vent_R™ DNA polymerase

- Reaction Buffer: 10X ThermoPol buffer
- DNA polymerase Stock: Deep Vent_R™ (exo-) DNA pol (2 U/ μ L); amount per reaction = 1 U (0.5 μ L)

Tth DNA polymerase

- Reaction Buffer: 10X PCR buffer
- DNA polymerase Stock: *Tth* DNA pol (5 U/ μ L); amount per reaction = 5.0 U (1 μ L)

Tfi DNA polymerase

- Reaction Buffer: 5X *Tfi* PCR Reaction Buffer
- Supplement with: 0.75 μ L of 50 mM $MgCl_2$; 1.5 mM $MgCl_2$
- DNA polymerase Stock: *Tfi* DNA pol (5 U/ μ L); amount per reaction = 5 U (1 μ L)

EconoTaq™ DNA Polymerase

- Reaction Buffer: 10X Reaction Buffer
- DNA polymerase Stock: EconoTaq™ DNA pol (5U/μL); amount per reaction = 2.5 U (0.5 μL)

Use Guidelines

1. Page 6 lists the enzymes we have validated for use with CleanAmp™ dNTPs. We have found *Taq*, both native and recombinant, to work well in all applications tested. CleanAmp™ dNTPs were also shown to successfully block extension by mesophilic enzymes, such as Klenow DNA polymerase.
2. PCR buffers with a pH range of 8 to 9 can be used for your PCR set-up.
3. For standard thermal cycling protocols, we recommend 2.5 mM MgCl₂, 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 MgCl₂ concentration should also be increased. For further recommendations, see pages 9-10 or contact our technical support team.
4. 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.
5. CleanAmp™ dNTPs are validated for amplicons up to 2 kb in length.
6. When using cDNA as your template, we recommend purifying the product using a commercially-available clean-up kit to remove unincorporated nucleotides. Should your protocol require the use of the cDNA product without purification, your cDNA synthesis product should be no more than 1/10th of the reaction volume of your PCR setup.
7. In multiplex reactions where four or more targets are amplified, the addition of KCl up to 100 mM final concentration will improve results. Be sure to take into account the KCl content of your PCR buffer in these optimizations.

Protocols for *Taq* DNA Polymerase

Standard Thermal Cycling: 25 μ L Endpoint PCR

Fast Thermal Cycling: 25 μ L Endpoint PCR

Multiplexed: 25 μ L Standard Thermal Cycling (2 or more targets)

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) to desired working concentration.
3. Make a mastermix containing all components except for the DNA template sample. Add each of the components as shown on page 10 (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 μ L of mastermix into each thin-walled PCR tube.
6. To each 20 μ L aliquot of mastermix, add 5 μ L of the appropriate template DNA for a final reaction volume of 25 μ L.
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 and multiplexed thermal cycling conditions:

95°C for 2-10 min
[95°C for 30 sec; 48-60°C¹ for 30 sec; 72°C for 0.5-2 min] 25-50 cycles
72°C for 10 min

For fast thermal cycling conditions:
[95°C for 10 sec; 62-72°C² for 30 sec] 40-50 cycles
9. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

Component	Final Concentration (in a 25 μ L reaction)	Volume for 1 reaction	Volume for 10 reactions
Forward/Reverse Primer	50-500 nM	Variable	Variable
Sterile de-ionized water	Up to 20 μ L	Up to 20 μ L	Up to 200 μ L
50 mM Magnesium Chloride			
Standard Thermal Cycling ³	2.5 mM	1.25 μ L	12.5 μ L
Fast Thermal Cycling	4.0 mM	2 μ L	20 μ L
Multiplexed ⁴	4.0 mM	2 μ L	20 μ L
PCR buffer 10X⁵			
Standard Thermal Cycling ³	1X	2.5 μ L	25 μ L
Fast Thermal Cycling	1X	2.5 μ L	25 μ L
Multiplexed	1X	2.5 μ L	25 μ L
CleanAmp™ dNTP mixture (10 mM of each)			
Standard Thermal Cycling	0.4 mM	1 μ L	10 μ L
Fast Thermal Cycling	0.4 mM	1 μ L	10 μ L
Multiplexed	0.8 mM	2 μ L	20 μ L
Taq DNA polymerase (5/μL)			
Standard Thermal Cycling ³	0.05 units/ μ L	0.25 μ L	2.5 μ L
Fast Thermal Cycling	0.20 units/ μ L	1 μ L	10 μ L
Multiplexed	0.05-0.10 units/ μ L	0.25-0.5 μ L	2.5-5.0 μ L
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 Use the following calculation for the annealing/extension temperature:
 Annealing/extension temperature ($^{\circ}$ C) = (72 $^{\circ}$ C + average T_m of primers)/2

³ For Standard Thermal Cycling protocols utilizing alternate DNA polymerases, see pages 7-8 for specific parameters. The magnesium chloride concentration can be optimized between 2.5 and 4.0 mM.

⁴ In multiplex reactions where four or more targets are amplified, the addition of KCl up to 100 mM final concentration will improve results. Be sure to take into account the KCl content of your PCR buffer in these optimizations.

⁵ 10X PCR buffer for Taq DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25 $^{\circ}$ C; 500 mM KCl)

Real-Time

The standard, multiplexed and fast cycling protocols on pages 9-10 can be adapted for real-time experiments with the following alterations to the protocol:

Additional real-time components

SYBR® Green detection:

- o SYBR® Green I Nucleic Acid Stain -10,000X (Invitrogen)
- o Passive reference ROX dye - 1 mM (Agilent)

Hydrolysis probe detection

- o Hydrolysis probe
- o Passive reference ROX dye - 1 mM (Agilent)

Adaptation to real-time PCR: The reaction setup from below should be identical, except for the following additions for SYBR® Green- or hydrolysis probe-based detection. Reactions should be incubated in a thermal cycler capable of real-time detection, where fluorescence data should be collected at the completion of the annealing step of each cycle.

- o For SYBR® Green I-based detection, 30 or 300 nM passive ROX reference dye (dependent on real-time instrument manufacturer, please reference Agilent's manual for details) and 0.15X SYBR® Green I Nucleic Acid Stain should be included in the reaction.
- o For hydrolysis probe-based detection, 30 or 300 nM passive ROX reference dye (dependent on real-time instrument manufacturer, please reference Agilent's manual for details) 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 C_q and maximal fluorescence intensity.

Note: The dilution of passive ROX reference dye can be kept at 4°C for approximately one month. For both the passive ROX reference dye and TaqMan® Probe, 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:

Observation	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 magnesium 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 concentration).		
Non-specific product formation	Excessive off-target primer extension	Titrate the concentration of the primers or template DNA.
		Reduce the extension time to avoid spurious amplification products.
		Reduce the amount of DNA polymerase.

Contact Information

For further information on CleanAmp™ dNTPs, please visit our website or contact TriLink directly. We have an excellent technical support team available to help with any additional questions about this novel technology.

CleanAmp™ Products online:

www.trilinkbiotech.com/cleanamp

Contact TriLink:

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