CleanAmp™ dNTPs

The Next Generation in Hot Start PCR

Product Manual

TriLink BioTechnologies

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The Modified Nucleic Acid Experts

Contents

Materials Provided

Material	Catalog #	Quantity	Volume
CleanAmp™ dNTP Mix: dATP, dCTP, dGTP and dTTP each at 10 mM	N-9506-2	2 µmole each (4 x 2 µmoles)	1 x 200 µl
	N-9506-10	10 µmole each (4 x 10 µmoles)	1 x 1000 µl
CleanAmp™ dNTP Set: 1 (50 mM) vial each of dATP, dCTP, dGTP and dTTP	N-9507-2	2 µmole each (4 x 2 µmoles)	4 x 40 µl
	N-9507-10	10 µmole each (4 x 10 µmoles)	4 x 200 µl

Storage Conditions

Upon receipt, store at -20°C or below. Do not expose the stock solution to more than 24 total hours at room temperature.

CleanAmp™ Stock Solution Stability:

-20°C	4°C	Room Temperature
At Least 1 Year	15 Days	24 Hours

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 approaches, 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 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. 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 several Hot Start and non-Hot Start DNA polymerases that employ different buffer compositions, pH 7.5 up to pH 9, at 25°C

Details

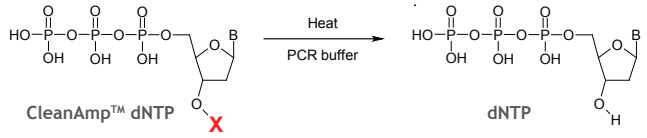
CleanAmp™ dNTPs are modified with a thermolabile protecting group (X) at the 3' terminus. The presence of this modification blocks DNA polymerase nucleotide incorporation until the nucleotide protecting group is removed using a heat activation step. When standard cycling protocols are employed, a 0-10 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.

Although we recommend using the CleanAmp™ dNTP Mix, which contains the modified nucleoside triphosphates of dA, dC, dG and dT, we have sometimes found that replacement of just one or two natural nucleotides with CleanAmp™ dNTPs is enough to have the desired effect. CleanAmp™ dNTPs are also available individually as a set. Simply substitute the natural dNTPs with one or more of the corresponding CleanAmp™ dNTPs. We have recommended optimal conditions for commonly-used applications (pages 6-11), however good results can be obtained with a wide variety of conditions.

Handling

Prior to Use

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.

Handling Guidelines

1. The CleanAmp™ dNTP Mix is shipped as a concentrated 10 mM solution of dATP, dCTP, dGTP and dTTP. CleanAmp™ dNTP Sets are shipped as a 50 mM solution. CleanAmp™ dNTPs 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 one year. CleanAmp™ dNTPs are very stable in the stock solution in which they are delivered.
2. CleanAmp™ dNTPs can be stored for up to 15 days at 4°C as the CleanAmp™ dNTP stock solution.
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. Do NOT thaw CleanAmp™ dNTPs by heating.

Product Use

CleanAmp™ dNTPs were designed as a replacement for natural nucleotides in reactions using standard thermophilic DNA polymerases such as *Taq* and *Pfu*. Table 1 lists the enzymes qualified for use with CleanAmp™ dNTPs using basic PCR primer template systems. 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 ~ 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. Although CleanAmp™ dNTPs improve PCR performance when used with standard primers and a non-Hot Start DNA polymerase, we have found a further benefit with other Hot Start reagents in some instances.

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
Taq	Enzymatics	8.3	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
EconoTaq®	Lucigen	9.0	5.0
Phusion®	Finnzymes	-	2.0

Use Guidelines

1. Table 1 lists the enzymes 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 from 8 – 9 can be used for your PCR setup.
3. 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. For further recommendations, see footnote 7 on page 8 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 µL Endpoint PCR
Fast Thermal Cycling: 25 µL Endpoint PCR
Multiplexed: 25 µL Standard Thermal Cycling (2 to 7 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. 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 on page 7 (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. 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:
   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
   For fast thermal cycling conditions:
   98°C for 30 sec [95°C for 5 sec; 65°C for 5 sec][2] 45 cycles
9. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

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)			
Standard Thermal Cycling[3]	2.5 mM	1.25 µL	12.5 µL
Fast Thermal Cycling	4.0 mM	2 µL	20 µL
Multiplexed[4]	2.5 mM	1.25 µL	12.5 µL
PCR buffer 10X[5]			
Standard Thermal Cycling[3]	1X	2.5 µL	25 µL
Fast Thermal Cycling	1X	2.5 µL	25 µL
Multiplexed	1X	2.5 µL	25 µL
CleanAmp™ dNTP solution[6,7]			
Standard Thermal Cycling	0.4 mM	1 µL	10 µL
Fast Thermal Cycling	0.4 mM	1 µL	10 µL
Multiplexed	0.4 mM	1 µL	10 µL
Taq DNA polymerase (5µ/µL)			
Standard Thermal Cycling[3]	0.05 units/µL	0.25 µL	2.5 µL
Fast Thermal Cycling	0.20 units/µL	1 µL	10 µL
Multiplexed	0.05-0.10 units/µL	0.25-0.5 µL	2.5-5.0 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] Use the following calculation for the annealing/extension temperature:
Annealing/extension temperature (˚C) = (72˚C + average Tm of primers)/2

[3] For Standard Thermal Cycling protocols utilizing alternate DNA polymerases, see page 9 for specific parameters. The $MgCl_2$ 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 Invitrogen's *Taq* DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25°C; 500 mM KCl)

[6] 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.

[7] 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, multiplexed and fast cycling protocols on pages 6-7 can be adapted for real-time experiments with the following alterations to the protocol:

Additional real-time components
SYBR® Green detection:
- SYBR® Green I Nucleic Acid Stain -10,000X (Invitrogen)
- Passive reference ROX dye - 1 mM (Agilent)

SYTO® 9 detection:
- SYTO® 9 Green Fluorescent Nucleic Acid Stain -5 mM (Invitrogen)
- Passive reference ROX dye - 1 mM (Agilent)

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

Adaptation to real-time PCR: The reaction setup should be identical to the setup on pages 6-7, 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 SYBR® Green I-based detection, 30 or 300 nM passive ROX reference dye and 0.15X SYBR® Green I Nucleic Acid Stain should be included in the reaction.
- 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.
- For hydrolysis probe-based detection, 30 or 300 nM passive ROX reference dye 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, TaqMan® 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.

Standard Thermal Cycling Conditions for Other DNA Polymerases

Taq DNA polymerase (Invitrogen) - 25 µL reaction
- Reaction Buffer: 10X PCR buffer
- Supplement with an additional 2.5 mM $MgCl_2$ (2.5 mM final concentration)
- _Taq_ DNA pol Stock (5 U/µL); amount per reaction = 1.25 U (0.25 µL)

Taq DNA polymerase (New England Biolabs) - 25 µL reaction
- Reaction Buffer: 10X Standard _Taq_ Reaction Buffer
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)
- _Taq_ DNA pol Stock (5 U/µL); amount per reaction = 1.25 U (0.25 µL)

Taq DNA polymerase (USB) - 25 µL reaction
- Reaction Buffer: 10X PCR Reaction Buffer
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)
- _Taq_ DNA pol Stock (5 U/µL); amount per reaction = 1.25 U (0.25 µL)

Taq DNA polymerase (Enzymatics) - 25 µL reaction
- Reaction Buffer: 10X PCR Reaction Buffer I
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)
- _Taq_ DNA pol Stock (5 U/µL); amount per reaction = 1.0 U (0.2 µL)

Pfu (exo+) and (exo-) DNA polymerase - 100 µL reaction
- Reaction Buffer: 10X Cloned _Pfu_ buffer
- **DNA polymerase Stock:**
  Cloned _Pfu_ (exo+) DNA pol (2.5 U/µL); amount per reaction = 2.5 U (1.0 µL)
  Cloned _Pfu_ (exo-) DNA pol (2.5 U/µL); amount per reaction = 2.5 U (1.0 µL)

DyNAzyme™ II DNA polymerase - 50 µL reaction
- Reaction Buffer: 10X Optimized DyNAzyme™ Buffer
- DyNAzyme™ II DNA pol Stock (2 U/µL); amount per reaction = 2 U (1.0 µL)

Deep Vent$_R$™ (exo-) DNA polymerase - 100 µL reaction
- Reaction Buffer: 10X ThermoPol buffer
- Deep Vent$_R$™ (exo-) DNA pol Stock (2 U/µL); amount per reaction = 1.25 U (1.0 µL)

Tth DNA polymerase - 25 µL reaction
- Reaction Buffer: 10X PCR buffer
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)

- _Tth_ DNA pol Stock (5 U/µL); amount per reaction = 5.0 U (1 µL)

Tfi DNA polymerase - 25 µL reaction
- Reaction Buffer: 5X _Tfi_ PCR Reaction Buffer
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)
- _Tfi_ DNA pol Stock (5 U/µL); amount per reaction = 5 U (1 µL)

EconoTaq™ DNA polymerase - 50 µL reaction
- Reaction Buffer: 10X PCR Reaction Buffer
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)
- EconoTaq™ Stock (5 U/µL); amount per reaction = 1 U (0.2 µL)

Phusion® High-Fidelity DNA polymerase - 50 µL reaction
- Reaction Buffer: 5X HF PCR Reaction Buffer
- Supplement with an additional 1.0 mM $MgCl_2$ (2.5 mM final concentration)
- Stock (2 U/µL); amount per reaction = 1 U (0.5 µL)

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 $MgCl_2$ 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 concentration).
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 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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