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Product Information

CleanAmp™ dNTPs

Catalog Numbers **DNTPCA1, DNTPCA2, DNTPCA10**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
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. They 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. They offer excellent results in a number of PCR-based applications for a fraction of the cost of other Hot Start solutions.

CleanAmp dNTPs are modified with a thermolabile protecting group (see Figure 1) 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.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store at –20 °C. Do not expose the stock solution to more than 24 hours at room temperature.

For best performance, we recommend distributing your stock solution into smaller aliquots that are sufficient for one week of work. To avoid prolonged exposure of the CleanAmp dNTP Mix stock solution to room temperature, store stock nucleotide solutions in the freezer at −20 °C. We do not recommend subjecting the CleanAmp dNTPs to more than ten freeze-thaw cycles.

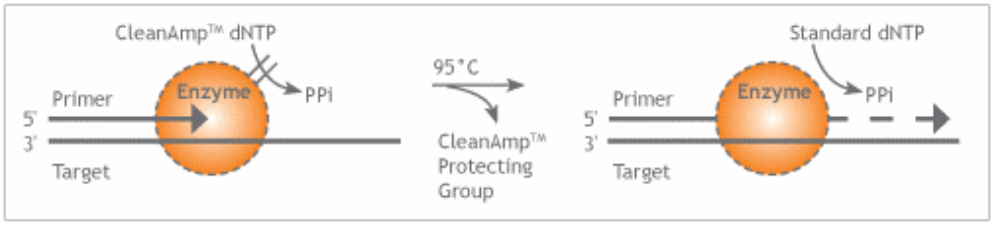

Figure 1.

Reagents Supplied

Material	Catalog #	Quantity	Volume
CleanAmp dNTP Mix: dATP, dCTP, dGTP and TTP, each at 10 mM	DNTPCA1-2UMOL	2 µmole each	1 x 200 µL
	DNTPCA1- 10UMOL	10 µmole each	1 x 1000 µL
CleanAmp dNTP Set: 1 vial of dATP, dCTP, dGTP and TTP, each at 50 mM	DNTPCA2-1KT	2 µmole each	4 x 40 µL
	DNTPCA10-1KT	10 µmole each	4 x 200 µL

Reagents and equipment required but not provided

- Taq DNA Polymerase, Catalog No. D4545 (supplied with 10X PCR Buffer without $MgCl_2$)
- $MgCl_2$ solution, 25 mM, Catalog No. M8787
- Water, PCR Reagent, Catalog Number W1754
- PurePak PCR microtubes, thin-walls, Catalog Numbers P3114 (0.2 mL) and P3364 (0.5 mL)
- Thermal cycler
- Primers
- DNA to be amplified

Procedure

Note: CleanAmp dNTPs were designed to be used as a replacement for natural nucleotides in reactions using standard thermophilic DNA polymerases such as *Taq*. 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. PCR performance is ideal over a primer concentration range of 0.05-0.5 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 below (multiply amounts by the number of reactions needed) in a centrifuge tube, on ice.

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
$MgCl_2$ (25 mM)	2.5 mM	2.5 µL	25 µL
10X PCR Buffer	1X	2.5 µL	25 µL
CleanAmp dNTP Solution	0.4 mM	1 µL	10 µL
Taq DNA Polymerase (5U/µL)	0.05 units/ µL	0.25 µL	2.5 µL
Total Volume (µL)	20 µL	20 µL	200 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 standard thermal cycling:

Initial denaturation	95 °C	0-5 min
30-40 cycles:		
Denaturation	95 °C	10 sec
Annealing	48 °C to 60 °C	1-30 sec
Extension	72 °C	0.5-2 min
Final extension:	72 °C	10 min

9. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

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, Catalog No. S9430) 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.

Label License Statement

NOTICE TO PURCHASER: DISCLAIMER OF LICENSE

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Troubleshooting Guide

Problem	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 complementary 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: 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:

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