CleanAmp™ dUTP

Catalog # N-9524

N-9524-2 (2 µmoles at 50 mM concentration)
N-9524-10 (10 µmoles at 50 mM concentration)

Sodium Salt form | Store at -20°C or below

QC Analysis

Functional Assay; Pass
Tested in standardized PCR assay

AX-HPLC; Pass RP-HPLC; Pass

^{31}P NMR; Pass Mass Spec; Pass

Product released by Quality Assurance

Product Overview

PCR amplification products can cause false positives in downstream detection. Employment of Uracil-N-glycosylase (UNG), which recognizes and removes uracil residues, is the most common method for carryover decontamination in PCR. This method also substitutes dUTP for dTTP during PCR, resulting in amplicons that are distinct from the DNA template.

TriLink's CleanAmp™ dUTP is a hot start version of this analog, preventing nucleotide incorporation until the heat activation step. The patented CleanAmp™ technology employs thermolabile protecting groups blocking DNA polymerase function until activation by heat or pH adjustment.

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

Substitute CleanAmp™ dUTP for dTTP. Optimal PCR results are achieved when CleanAmp™ dUTP is in three-fold excess of dATP, dCTP and dGTP. Use CleanAmp™ dUTP with standard dNTPs for clean targets and with CleanAmp™ dNTPs for problematic targets. CleanAmp™ dUTP and has been evaluated in routine PCR. See reverse for standard thermal cycling protocol and dNTP mixture preparation instructions. 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.

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 target amplification:
   95°C for 10 min
   [95°C for 40 sec; X°C[1] for 1-30 sec; 72°C for 0.5-2 min]
   35-40 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 25 µL	Up to 25 µL	Up to 250 µL
$MgCl_2$ (50 mM)[2]	4.5 mM	2.25 µL	22.5 µL
PCR buffer 10X[3]	1X	2.5 µL	25 µL
CleanAmp™ dUTP Mix[4]	0.2 mM	1 µL	10 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**

[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 3.0 and 5.0 mM.
[3] 10X PCR buffer for Invitrogen's *Taq* DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25°C; 500 mM KCl)
CleanAmp™ dUTP should be used in a 3-fold excess over dATP, dCTP and dGTP. See CleanAmp™ dUTP Mix Preparation below for the recommended nucleotide composition.

CleanAmp™ dUTP Mix Preparation

Table 1

Component	Stock Concentration[6]	Desired Concentration	Volume[6]
dATP[5]	50 mM	10 mM	4 µL
dCTP[5]	50 mM	10 mM	4 µL
dGTP[5]	50 mM	10 mM	4 µL
CleanAmp™ dUTP	50 mM	30 mM	12 µL
Sterile Deionized Water	-	-	16 µL
			40 µL

[5] Standard or CleanAmp™ dNTPs can be used.
[6] Typical stock concentrations shown. Adjust volumes accordingly, as needed.

Troubleshooting

- Should excess banding be seen; try using less DNA polymerase.
- The annealing temperature should be chosen for optimal PCR performance.
- 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 $MgCl_2$.
- Use CleanAmp™ dUTP (N-9524) with standard dNTPs (N-2505) for clean targets or with CleanAmp™ dNTPs (N-9507) for more problematic targets.
- Titrate template or primer concentrations for better specificity.

Contact

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