Biotin-dCTP

γ-[N-(Biotin-6-amino-hexanoyl-6-aminobutanoyl)]-5-(3-propargylamino)-2′-deoxy-cytidine-5′-triphosphate, triethylammonium salt

Instruction Manual

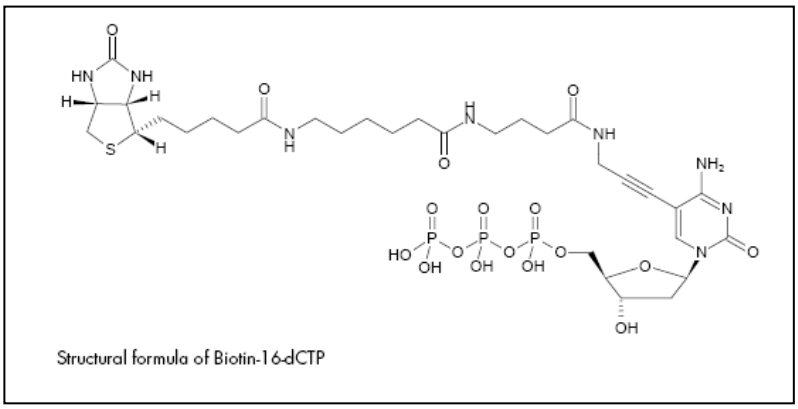
Product Name	Product Description	Size	Catalog Number
Biotin-dCTP	Biotin-conjugated propagylamino-dCTP (1 mM)	200 µl (1 mM) 1 ml (1 mM)	PK-CA-BIO-DCTP-200P PK-CA-BIO-DCTP-1000P

Product Description

Biotin-dCTP is recommended for direct enzymatic labeling of DNA. The labeled-dCTP is optimized for incorporation into DNA/cDNA by a variety of enzymes. Recommended labeling methods include PCR with Taq Polymerase, Nick Translation with DNAse I/ DNA Polymerase, Primer Extension with Klenow *exo*$^-$, Reverse Transcription with MMLV Reverse Transcriptase, and 3'-end labeling with Terminal deoxynucleotidyl Transferase (TdT).
Biotin-16-dCTP is enzymatically incorporated into DNA/cDNA as substitute for its natural counterpart dCTP. The resulting Biotin-labeled DNA/cDNA probes are subsequently detected using streptavidin conjugated with horseradish peroxidase (HRP), alkaline phosphatise (AP), a fluorescent dye or agarose/magnetic beads. Optimal substrate properties and thus labeling efficiency as well as an efficient detection of the Biotin moiety is ensured by a 16-atom linker attached to the C5 position of cytidine.
In PCR labeling, repeated cycles of denaturation, annealing and extension allow the amplification of a specific DNA fragment. Extension of the annealed primers with Taq polymerase results in a duplication of the DNA fragment in each cycle. When dCTP is partially substituted by Biotin-dCTP a Biotin labeled double-stranded DNA is generated.
The resultant DNA is suited for a variety of hybridization experiments, including Southern and Northern blots, colony hybridizations and in situ hybridizations.

Specifications

Molecular Formula: $C_{32}H_{51}N_8O_{17}P_3S$ (free acid)
Molecular Weight: 944.78 g/mol (free acid)
Purity: ≥95 % (as determined by HPLC)
Form: sterile-filtered, clear aqueous solution, in 10 mM Tris-HCl (pH 7.5)
Concentration: 1 mM
pH: 7.5
Spectroscopic Properties: λmax = 294 nm; ε = 9.3 l mmol^{-1} cm^{-1}
(Tris-HCl, pH 7.5)



Structural formula of Biotin-16-dCTP

Storage and Stability

Store at -20°C. Avoid frequent thawing and freezing.

Labeling Protocol

Recommended PCR assay

20 µl PCR labeling assay

Component	Stock conc.	Amount	Final conc.
High yield buffer without $MgCl_2$	10x	2 µl	1x
$MgCl_2$ stock solution	25 mM	1.6 µl	2 mM
dATP	1 mM	2 µl	100 µM
dCTP	1 mM	1 µ l [1)]	50 µM [1)]
dGTP	1 mM	2 µl	100 µM
dTTP	1 mM	2 µl	100 µM
Biotin-dCTP	1 mM	1 µl [1)]	50 µM [1)]
forward Primer	10 µM	1 µl	500 nM
reverse Primer	10 µM	1 µl	500 nM
Template DNA		0.1-10 ng	5-500 pg/µl
Taq Polymerase	5 units/µl	0.2 µl (1 unit)	0.05 units/µl
PCR grade H_2O		Fill up to 20 µl	

[1)] Recommended Biotin-16-dCTP/dCTP ratio for PCR: 50% Biotin-16-dCTP/ 50% dCTP. The optimal final concentration of the labeled nucleotide may vary depending on the application.

Recommended cycling conditions

Initial denaturation	94°C	2 min	1x
Denaturation	94°C	30 sec	25-30x
Annealing [1)]	50-60°C	30 sec	
Elongation [2)]	72°C	1 min	
Final elongation	72°C	5 min	1x

1. The annealing temperature depends on the melting temperature of the primers used.
2. The elongation time depends on the length of the fragments to be amplified. A time of 2 min/kbp is recommended.

Please note: For optimal amplification results and high incorporation rates an individual optimization of the Biotin-16-dCTP/dCTP ratio, the recommended PCR assay and the cycling conditions may be necessary for each new primer-template pair.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

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