

## Instruction Manual

Product Name	Product Description	Size	Catalog Number
Biotin-dUTP	Biotin-conjugated aminoallyl-dUTP (1 mM)	200 µl (1 mM) 1 ml (1 mM)	PK-CA-BIO-DUTP-200P PK-CA-BIO-DUTP-1000P

### Product Description

Biotin-dUTP is recommended for direct enzymatic labeling of DNA. The labeled-dUTP 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*<sup>-</sup>, Reverse Transcription with MMLV Reverse Transcriptase, and 3'-end labeling with Terminal deoxynucleotidyl Transferase (TdT).

Biotin-16-dUTP is enzymatically incorporated into DNA/cDNA as substitute for its natural counterpart dTTP. The resulting Biotin-labeled DNA/cDNA probes are subsequently detected using streptavidin conjugated with horseradish peroxidase (HRP), alkaline phosphatase (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 uridine.

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 dTTP is partially substituted by Biotin-dUTP 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<sub>32</sub>H<sub>52</sub>N<sub>7</sub>O<sub>18</sub>P<sub>3</sub>S (free acid)

**Molecular Weight:** 947.78 g/mol (free acid)

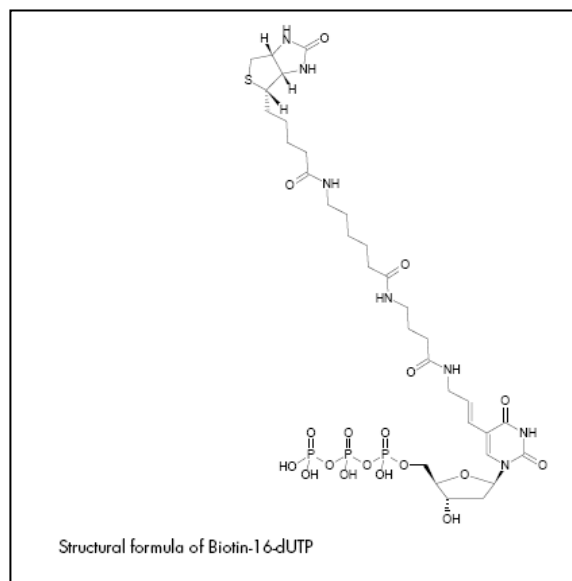
**Purity:** ≥98 % (as determined by HPLC)

**Form:** sterile-filtered, clear aqueous solution (pH 7.5)

**Concentration:** 1 mM

**pH:** 7.5

**Spectroscopic Properties:** λ<sub>max</sub> = 240 nm; ε = 10.7 l mmol<sup>-1</sup> cm<sup>-1</sup> (Tris-HCl, pH 7.5)



### 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 <sub>2</sub>	10x	2 µl	1x
MgCl <sub>2</sub> stock solution	25 mM	1.6 µl	2 mM
dATP	1 mM	2 µl	100 µM
dCTP	1 mM	2 µl	100 µM
dGTP	1 mM	2 µl	100 µM
dTTP	1 mM	1 µl	50 µM
Biotin-dUTP	1 mM	1 µl <sup>1)</sup>	50 µM <sup>1)</sup>
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 <sub>2</sub> O		Fill up to 20 µl	

<sup>1)</sup> Recommended Biotin-16-dUTP/dUTP ratio for PCR: 50% Biotin-16-dUTP/ 50% dTTP. 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 <sup>1)</sup>	50-60°C	30 sec	
Elongation <sup>2)</sup>	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-dUTP/dTTP 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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