Biotin PCR Labeling Kit

PromoKine

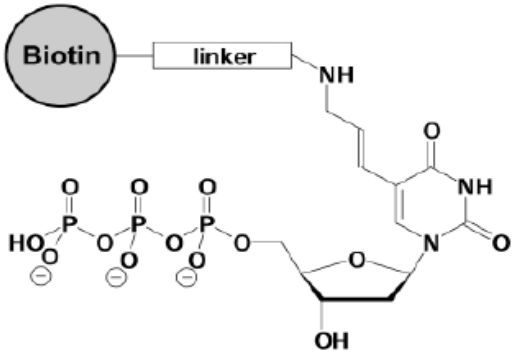
Instruction Manual

Product Name	Product Description	Size	Catalog Number
Biotin PCR Labeling Kit	Biotin PCR Labeling Kit	100 reactions 500 reactions	PK-CA-BIO-LK100 PK-CA-BIO-LK500

Product Description

The Biotin PCR Labeling Kit contains all reagents required for PCR labeling providing a highly efficient, easy-to-perform and rapid labeling technology. The kit is recommended for direct enzymatic labeling of DNA. The Biotin PCR labeling mix contains specially optimized Biotin-dUTP for incorporation into DNA by PCR using Taq polymerase. In PCR labeling, repeated cycles of denaturation, annealing and extension allow the amplification of a specific DNA fragment. When dTTP is partially substituted by dye-dUTP the extension of the annealed primers with Taq polymerase generates fluorescent labeled double-stranded DNA. The resultant DNA is suited for a variety of hybridization experiments, including Southern and Northern blots, colony hybridizations and *in situ* hybridizations.

Structure



Biotin-dUTP. Biotin is attached via an optimized linker to aminoallyl-dUTP.

Kit Contents

Kits content reagents for 100 labeling reactions.

- Taq Pol (red cap): 2 units/µl Taq Polymerase in storage buffer
- 10x PCR labeling buffer (green cap): 10x concentration
- Biotin PCR labeling mix (purple cap): 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.5 mM dTTP, 0.5 mM Biotin-dUTP, pH 7.5
- PCR grade water (white cap)

Storage and Stability

Store at -20°C, avoid frequent thawing and freezing. Stable for up to 12 months under proper storage conditions. For *in vitro* use only.

Labeling Protocol

Recommended PCR assay

Prepare the following reaction mixture in a sterile vial, adding the enzyme last.

20 µl PCR labeling assay

to 20 µl final volume	PCR grade H_2O	white cap
2 µl	10x PCR labeling buffer	green cap
2 µl	Biotin PCR labeling mix	purple cap
1 µl	forward Primer (10 µM)	
1 µl	reverse Primer (10 µM)	
0.1-10 ng	Template DNA	
0.5 µl (1 unit)	Taq Pol	red cap

Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction at the bottom of the tube. Place the tube in a thermocycler.

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

For optimal amplification results and high incorporation rates, individual optimization of the recommended assay and cycling conditions may be necessary for each new primer-template pair.

Purification of the probe

To remove unincorporated nucleotides from the reaction mixture prior to its use in subsequent experiments one of the following procedures is recommended:

1. Purification by silica-gel membrane adsorption

- PromoKine PCR Purification Kit, Cat.-No. PK-MB20-300

The PromoKine PCR Purification Kit provides a simple and efficient way to purify DNA fragments larger than 100 bp. The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. Please refer to the instruction manual.

2. Purification by Centrifugal Filter Units

Unincorporated nucleotides can be removed by centrifugation using centrifugal filter units. Select the filter unit by its cut-off for DNA fragments and follow the manufacturer's instructions.

Intended Use

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

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