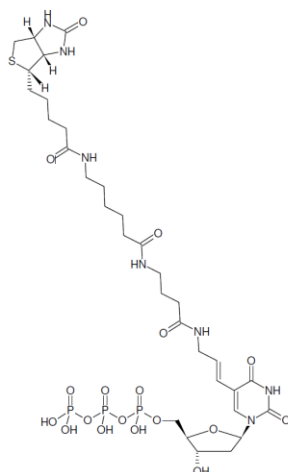
HighFidelity 16 PCR Labeling Kit (Biotin)

Cat #: orb653771 (manual)



Structural formula of HighFidelity Biotin16 PCR Labeling Kit

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Description:

HighFidelity Biotin16 PCR Labeling Kit is designed to produce randomly Biotin16-modified DNA probes by PCR. Such probes are ideally suited for *in situ* hybridization and Northern Blot experiments. PCR-based labeling is superior to random-primed labeling by Klenow fragment if template amounts are limited or amplification of a specific DNA fragments is required.

Biotin-16-dUTP is efficiently incorporated into DNA as substi- tute for its natural counterpart dTTP using an optimized reaction buffer and a High Fidelity Polymerase. 50 % Biotin-16-dUTP substi- tution typically results in an optimal balance between reaction and labeling efficiency. Individual optimization of Biotin-16-dUTP/dTTP ratio however, can easily be achieved with the single nucleotide format. The resulting Biotin16-modified DNA probe can subsequently be detected by HRP- or AP-modified Streptavidin.

The kit contains sufficient reagents for 175 labeling reactions of 20 µl each (50 % Biotin16-dUTP substitution, 100 µM dATP/dGTP/dCTP, 50 µM dTTP, 50 µM Biotin16-dUTP).

Content:

High Fidelity Polymerase

in storage buffer with 50% glycerol (v/v) 1x 200 µl (500 units, 2.5 units/µl)

High Fidelity Labeling Buffer

1x 500 µl (10x)

dATP - Solution

1x 20 µl (100 mM)

dGTP - Solution

1x 20 µl (100 mM)

dCTP - Solution

1x 20 µl (100 mM)

dTTP - Solution

1x 20 µl (100 mM)

Biotin-16-dUTP

1x 200 µl (1 mM)

Lambda DNA

1x 20 µl (100 ng/µl)

500 bp forward primer

1x 20 µl (10 µM)

500 bp reverse primer

1x 20 µl (10 µM)

PCR-grade water

1x 1.2 ml

To be provided by user

DNA template Primer

DNA purification tools (optional)

1. Preparation of working solutions

1.1 Preparation of 1 mM dATP/dCTP/dGTP working solution

- Thaw 100 mM dATP, 100 mM dCTP and 100 mM dGTP solutions on ice, voretex and spin-down briefly.
- Prepare a 1:100 dilution with PCR-grade water to achieve a final concentration of 1 mM (e.g. 2 µl 100 mM dATP + 2 µl 100 mM dCTP

  + 2 µl 100 mM dGTP + 194 µl PCR-grade water).
- 1 mM ATP/CTP/GTP working solution can be stored at -20°C. Pre- pare aliquots to avoid freeze/thaw cycles.

1.2 Preparation of 1 mM dTTP working solution

- Thaw 100 mM dTTP solution on ice, voretex and spin-down briefly.

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- Prepare a 1:100 dilution with PCR-grade water to achieve a final concentration of 1 mM (e.g. 2 µl 100 mM dTTP + 198 µl PCR-grade water).
- 1 mM dTTP working solution can be stored at -20 °C. Prepare aliquots to avoid freeze/thaw cycles.

2. Standard PCR Labeling protocol

The standard protocol is set-up for labeling of a 500 bp DNA fragment. An optimal balance between reaction and labeling efficiency is typically achieved with 50% Biotin-16-dUTP substitution following the standard protocol below however, individual optimiza- tion might improve results for individual applications.

- Assemble the PCR on ice in the order stated below (DNAse-free reaction tube).
- Voretex and spin-down briefly.

Component	Volume	Final concentration
PCR-grade water	X µl	
High Fidelity Buffer (10x)	2 µl	1x
1 mM dATP/dCTP/ dGTP working so- lution (s. 1.1)	2 µl	100 µM
1 mM dTTP working solution (s. 1.2)	1 µl	50 µM
1 mM Biotin-16- dUTP	1 µl	50 µM
forward primer (10 µM)	X µl	0.1 - 1 µM (e.g. 0.3 µM 500 bp forward primer)
reverse primer (10 µM)	X µl	0.1 - 1 µM (e.g. 0.3 µM 500 bp reverse primer)
template DNA	X µl	1 - 10 ng genomic DNA (e.g. 1 ng Lambda DNA)
High Fidelity Pol (2.5 units/µl)	1 µl	2.5 units
Total volume	20 µl	

Recommended cycling conditions

Cycle step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1x
Denaturation Annealing1) Elongation2)	95°C 58°C 68°C	20 sec 30 sec 60 sec	30x
Final Elongation	68°C	2 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. Elongation at 72°C works as well.

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

3. Probe purification:

Probe purification is not required for most hybridization experiments. If a downstream application requires purification (e.g. concentration determination by absorbance measurement) we recommend silica-membrane or gel filtration-based purification.

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