

Biotin-High Prime

For the nonradioactive labeling of DNA with biotin-16-dUTP using random oligonucleotides as primers. Premixed solution for 25 labeling assays.

Cat. No. 1 585 649

100 µl

Version 1, April 2000

Store at -15 to -25° C

1. Product overview

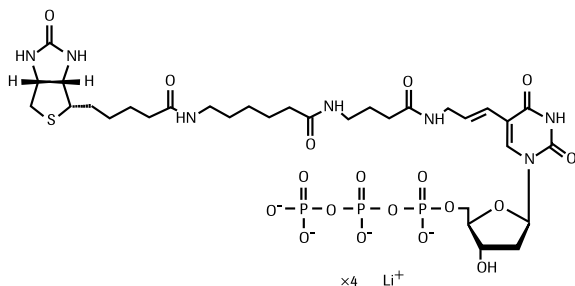
Contents

Cat. No.	Label	Content
1 585 649	Biotin-High Prime labeling mixture, 5x conc.	<ul style="list-style-type: none">• 100 µl• 5x conc.: random primer mix: 1 U/µl Klenow polymerase, labeling grade, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM biotin-16-dUTP, and 5x stabilized reaction buffer in 50% (v/v) glycerol

Labeling principle Biotin-labeled DNA probes are generated with Biotin-High Prime according to the "random primed" labeling technique (1,2). The complementary DNA strand is synthesized by Klenow polymerase using the 3'OH termini of the random oligonucleotides as primers. Biotin-16-dUTP (Fig. 1) is incorporated into the newly synthesized complementary DNA strand.

Biotin-High Prime is a specially developed reaction mixture* containing random oligonucleotides, Klenow polymerase, biotin-16-dUTP, dATP, dCTP, dGTP, dTTP, and an optimized reaction buffer concentrate in 50% glycerol for convenient and efficient nonradioactive labeling of DNA with biotin.

Figure 1: Structure of Biotin-16-dUTP



Application Biotin-High Prime labeled probes are used in a variety of hybridization techniques:

- Southern blots (3),
- Northern blots (4),
- Dot/slot blots
- screening of gene libraries (5),
- *in situ* hybridizations.

Sample material

- DNA fragments of at least 100 bp,
- linearized plasmids, cosmid or λDNA,
- supercoiled DNA,
- or minimal amounts of DNA (10 ng), e.g. DNA restriction fragments isolated from gels or in molten agarose can be used.

Number of labeling reactions 25 labeling reactions containing 0.01 -3 µg DNA can be performed with Biotin-High Prime.

Storage/Stability The unopened vial is stable at -15 to -25°C through the expiration date printed on the label.

Note: Repeated freezing and thawing should be avoided. To avoid contamination we recommend to aliquot the Biotin-High Prime solution and to store in 2-3 portions.

Detection of biotin-labeled DNA

Biotin-labeled DNA is detected by streptavidin conjugated to the enzyme alkaline phosphatase which catalyzes a color reaction with 5-bromo-4-chloro-3-indolyl-phosphatase and 4-nitro blue tetrazolium chloride (NBT/BCIP)* or by a chemiluminescent reaction with CSPD* or CDP-Star*.

- Alternatively, especially for *in situ* applications, biotin-labeled hybrids can also be detected by streptavidin conjugated to different fluorochromes
 - Avidin-AMCA*
 - Avidin-Fluorescein*
 - Avidin-Rhodamin 101*
- Biotin-labeled DNA can also be detected together with digoxigenin- and fluorescein-labeled DNA by Multicolor Detection Set*.

2. Procedures and required materials

2.1 Standard labeling assay

Additional equipment and reagents required

- water bath
- ice/water
- 0.2M EDTA (pH 8.0)

Procedure

In the following table please find a protocol for the standard labeling assay.

Step	Action
1	Add 1 µg template DNA (linear or supercoiled) and sterile, double dist. water to a final volume of 16 µl to a reaction vial.
2	Denature the DNA by heating in a boiling water bath for 10 min and chilling quickly in an ice/water bath. Note: Complete denaturation is essential for efficient labeling.
3	<ul style="list-style-type: none">• Mix Biotin-High Prime thoroughly and add 4 µl to the denatured DNA, mix, and centrifuge briefly.• Incubate for 1 h or O/N at 37°C.• Note: Longer incubations (up to 20 h) will increase the yield of Biotin-labeled DNA (see table below).
4	Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or by heating to 65°C for 10 min. Note: The length of the Biotin labeled fragments obtained with Biotin-High Prime range from 200 bp to 1000 bp or larger, depending on the lengths of the original template.

2.2 Labeling assay using low-melting point agarose

Procedure

In the following table the procedure for the labeling of DNA isolated from low-melting point agarose is described.

Step	Action
1	Excise the DNA fragment to be labeled cleanly from a low-melting point agarose gel and transfer it to a 1.5 ml microfuge tube.
2	<ul style="list-style-type: none"> Add sterile, double dist. water to a ratio of 3 ml/g gel and heat the tube for 7 min at 100°C to melt the gel and denature the DNA. After cooling to 37°C the DNA/agarose mixture can be used directly for labeling.
3	Mix Biotin-High Prime thoroughly and add 4 µl, mix, and centrifuge briefly.
4	Incubate overnight at 37°C.
5	Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or by heating to 65°C for 10 min.

3. Efficiency of Biotin-High Prime Labeling

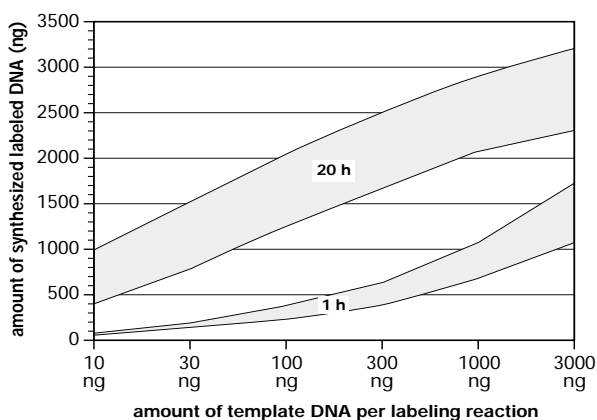
Table 1:

Yield of Biotin-High Prime labeling reaction:
Using the Biotin-High Prime solution labeling reactions were performed with increasing amounts of different template DNAs for 1 h and 20 h. The yield of biotin-labeling DNA was determined by the incorporation of a radioactive tracer and confirmed by a dot blot. (Average of independent labeling assays).

Template DNA	1 h	20 h
10 ng	70 ng	850 ng
30 ng	160 ng	1350 ng
100 ng	350 ng	1650 ng
300 ng	700 ng	2200 ng
1000 ng	1250 ng	2600 ng
3000 ng	1600 ng	2600 ng

Figure 2:

Yield of biotin-labeled DNA from different amounts of template DNAs for 1 h and 20 h incubation of the Biotin-High-Prime reaction at 37°C.



4. Appendix

4.1 References

- 1 Feinberg, A.P. & Vogelstein, B. (1983) *Anal. Biochem.* 132, 6.
- 2 Feinberg, A.P. & Vogelstein, B. (1984) *Anal. Biochem.* 137, 266.
- 3 Southern, E.M. (1975) *J. Mol. Biol.* 98, 503.
- 4 Smith, G.E. & Summers, M.D. (1990) *Anal. Biochem.* 109, 123.
- 5 Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* 72, 3961.

4.2 Related products

Kits

Product	Pack Size	Cat. No
Multicolor Detection Set	1 set 3x 50 substrate tablets	1 465 341
DIG Wash and Block Buffer Set	30 blots (10 x 10 cm ²)	1 585 762

Single reagents

Product	Pack Size	Cat. No.
Anti-biotin antibody	100 µg	1 426 320
Anti-biotin AP, Fab fragments	150 U	1 426 338
Anti-mouse-Ig-biotin	600 µg	821 462
Anti-mouse-Ig-biotin F(ab') ₂ fragments	for 20 000 tests	1 047 523
Avidin-AMCA	1 mg	2 012 987
Avidin-Fluorescein	1 mg	1 975 595
Avidin-Rhodamin	1 mg	1 975 609
Biotin-High Prime	100 µl	1 585 649
CDP-Star [®]	1 ml 2x 1 ml	1 685 627 1 759 051
CSPD [®] , ready-to use	2x 50 ml	1 755 633
DIG-High Prime	160 µl	1 585 606
High Prime	200 µl	1 585 592
Hybridization bags	50 bags	1 666 649
Klenow Enzyme	100 units 500 units	1 008 404 1 008 412
NBT/BCIP Stock Solution	8 ml	1 681 451
Nylon Membrane, positively charged (20 x 30 cm)	10 sheets	1 209 272
(10 x 15 cm)	20 sheets	1 209 299
(0.3 x 3 m roll)	1 roll	1 417 240

* available from Roche Molecular Biochemicals

¹CSPD and ²CDP-Star are trademarks of Tropix, Inc. Bedford, MA, USA and covered by European patent application 0 497 972 and US patent 5 112 960 and 5 326,882, both assigned to Tropix Inc. US

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