DIG-High Prime

For the nonradioactive labeling of DNA with DIG-11-dUTP alkali-labile, using random oligonucleotides as primers. Premixed solution for 40 labeling assays.

Cat. No. 11 585 606 910

160 µl

Version 09

Content version: August 2018:

Store at −15 to −25° C

1. Product overview

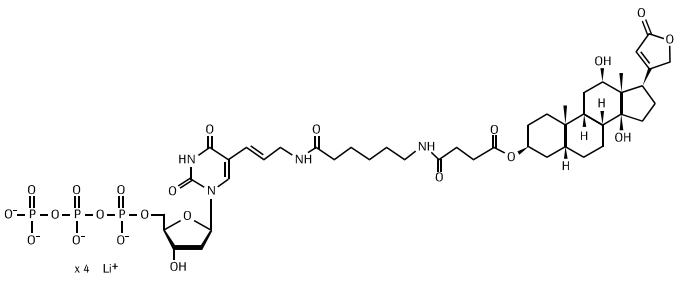
Contents

Cat. No.	Label	Content
11 585 606 910	DIG-High Prime labeling mixture, 5 × conc.	• 160 µl • 5 × conc. random primer mix: 1U/µl Klenow polymerase, labeling grade, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM DIG-11-dUTP, alkali labile, and 5 × stabilized reaction buffer in 50% (v/v) glycerol.

Labeling principle

DIG-labeled DNA probes are generated with DIG-High Prime according to the random primed labeling technique (1,2). DIG-High Prime is a specially developed reaction mixture* containing digoxigenin-11-dUTP (Fig.1) and all reagents necessary for random primed labeling, including Klenow enzyme, premixed in an optimized 5 × reaction buffer concentrate in 50% glycerol. The premixed DIG-High Prime reduces pipetting steps and increases yield, reproducibility, and convenience.

Fig. 1 Structure of alkali-labile DIG-11-dUTP



Application

DIG-High Prime labeled probes are used in a variety of hybridization reactions:

- 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 plasmid, cosmid or λ DNA
- supercoiled DNA
- or minimal amounts of DNA (10 ng), e.g. DNA restriction fragments isolated from gels or in molten agarose

Number of labeling reactions

Sufficient for 40 labeling reactions containing 0.01-3 µg DNA.

Storage/Stability

The unopened vial is stable at -15 to -25° C until the expiration date printed on the label.

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

Detection of DIG-labeled DNA

- DIG-labeled DNA is detected by an anti-DIG-AP, Fab fragments* which catalyzes a color or a chemiluminescent reaction. Special kits are available for color detection (DIG Nucleic Acid Detection Kit*), for chemiluminescent detection (DIG Luminescent Detection Kit*).
- Alternatively, especially for *in situ* applications, DIG-labeled hybrids can also be detected by anti-DIG antibodies conjugated to different fluorochromes.

Template DNA

The following table lists the recommended features of the template DNA

Feature	Detail
Purity	Template DNA should be prepared with the High Pure Plasmid Isolation Kit *. When other commercially available purification kits are used, we recommend to do an additional phenol/chloroform extraction to remove residual protein. This step is also necessary when templates have been treated with restriction or other modifying enzymes before labeling.
Size	To obtain optimal results, template DNA should be linearized and should have a size of ≧100 bp or larger. Template DNA >5 kb should be restriction-digested using a 4 bp cutter prior to labeling.
Amount	With the procedure described below principally 10 ng – 3 µg of template can be labeled, however, please check in the given table the necessary amount of probe needed for your size of blot. By scaling up of all volumes and components accordingly this procedure can be used for labeling of larger amounts. If single-copy gene detection in complex genomes is performed at least 300 ng of template DNA (probe concentration: 25 ng/ml hybridization solution) should be labeled.

Labeling of DNA isolated from agarose

If you intend to perform genomic Southern blotting, you should separate the template insert DNA from the vector by agarose gel electrophoresis.

To isolate DNA from the gel, you can use the Agarose Gel DNA Extraction Kit * for DNA fragments in the range of 400 bp to 5 kbp. It is applicable for standard agarose gels as well as low melting point agarose gels. Afterwards, the DNA fragments are efficiently labeled with digoxigenin without further purification. However, labeled probes should be purified with the High Pure PCR Product Purification Kit* to remove residual agarose particles.

2. Procedures and required materials

2.1 Standard labeling assay

Additional equipment and reagents required

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

Procedure

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

Step	Action
1	Add 1µg **template DNA** (linear or supercoiled) and autoclaved, double distilled 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 quickly chilling in an ice/water bath. ***Note***: Complete denaturation is essential for efficient labeling.
3	• Mix **DIG-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 DIG-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 DIG-labeled fragments obtained with DIG-High Prime range from 200 bp to 1000 bp or larger, depending on the lengths of the original template.

3. Efficiency of DIG-High Prime labeling

Table 1

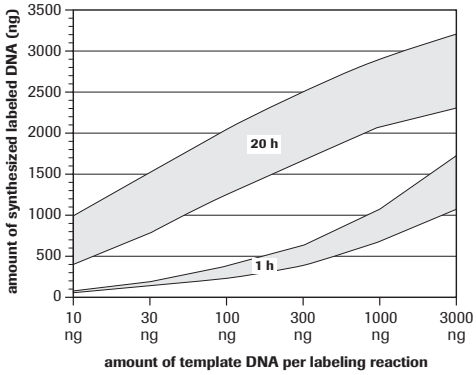
Yield of DIG-High Prime labeling reaction

Using the DIG-High Prime solution labeling reactions were performed with increasing amounts of different template DNAs for 1 h and 20 h. The yield of DIG-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	45 ng	600 ng
30 ng	130 ng	1050 ng
100 ng	270 ng	1500 ng
300 ng	450 ng	2000 ng
1000 ng	850 ng	2300 ng
3000 ng	1350 ng	2650 ng

Fig. 2

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



3.1 Semi-quantitative determination of labeling efficiency

Introduction

Determination of the yield of DIG-labeled DNA is most important for optimal and reproducible hybridization results. Too high of a probe concentration in the hybridization mix causes background, while too low of a concentration leads to weak signals.

Test principle

The preferred method for quantification of labeled probes is the direct detection method.

Stage	Description
1	• A series of dilutions of DIG-labeled DNA is applied to a small strip of nylon membrane positively charged*. • Part of the nylon membrane is preloaded with defined dilutions of DIG-labeled control DNA which are used as standards.
2	• The nylon membrane is subjected to immunological detection with anti-digoxigenin-AP conjugate* and CSPD ready-to-use*. • The intensities of the dilution series of DIG-labeled DNA and control DNA are compared by exposure to imaging device or X-ray film.

Probe quantification

Labeled probes and the DIG-labeled control DNA* must be diluted to 1 ng/µl, according to the expected yield of synthesized nucleic acid to start the dilution series below. The yield depends on the starting amount of template and incubation time.

Note: The yields given in table 1 where achieved under optimal conditions with highly purified template DNA.

Dilution series

Prepare a dilution series of your labeled probe and your control DNA as described in the table: (Procedure and working solutions are described in the pack insert of DIG-High Prime DNA Labeling and Detection Starter Kit II* in detail.)

Tube	DNA (µl)	From tube #	DNA Dilution Buffer (µl)	Dilution	Final concentration
1		original			1 ng/µl
2	5	1	495	1:100	10 pg/µl
3	15	2	35	1:3.3	3 pg/µl
4	5	2	45	1:10	1 pg/µl
5	5	3	45	1:10	0.3 pg/µl
6	5	4	45	1:10	0.1 pg/µl
7	5	5	45	1:10	0.03 pg/µl
8	5	6	45	1:10	0.01 pg/µl
9	0	-	50	-	0

Analyzing the results

Compare the intensity of the spots out of your labeling reaction to the control and calculate the amount of DIG-labeled DNA. If the 0.1 pg dilution spots of your probe and of the control are visible, then the labeled probe has reached the expected labeling efficiency (pls. see table 1) and can be used in the recommended concentration in the hybridization.

4. Appendix

4.1 References

1 Feinberg, A.P. & Vogelstein, B.(1983) *Anal. Biochem.* **132**, 6.
2 Feinberg, A.P. & Vogelstein, B.(1984) *Anal. Biorhem.* **137**, 266.
3 Southern, E.M. (1975) *J. Mol. Biol.* **98**, 503.
4 Srnith, 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 Ordering Information

Kits

Product	Pack Size	Cat. No
DIG-High Prime Starter Kit I	1 Kit (12 labeling and 24 detection reactions)	11 745 832 910
DIG-High Prime Starter Kit II	1 Kit (12 labeling and 24 detection reactions)	11 585 614 910
DIG Chemiluminescent Detection Kit	1 Kit	11 363 514 910
DIG Nucleic Acid Detection Kit	1 Kit	11 175 041 910
DIG Wash and Block Buffer Set	30 blots (10×10 cm^2)	11 585 762 001
High Pure Plasmid Isolation Kit	1 kit	11 754 777 001
Agarose Gel DNA Extraction Kit	1 kit	11 696 505 001
High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001

Single reagents

Product	Pack Size	Cat. No.
Anti-DIG AP, Fab fragments	150 U	11 093 274 910
Anti-Fluorescein-AP, Fab fragments	150 U	11 426 338 910
Biotin-High Prime	100 µl	11 585 649 910
CSPD	1 ml	11 655 884 001
CSPD, ready-to-use	2×50 ml	11 755 633 001
CDP-*Star*, ready-to-use	2×50 ml	12 041 677 001
DIG Easy Hyb	500 ml	11 603 558 001
DIG Easy Hyb Granules	6×100 ml	11 796 895 001
DIG-labeled control DNA	50 µl	11 585 738 910
Hybridization bags	50 bags	11 666 649 001
Klenow Enzyme	100 units 500 units	11 008 404 001 11 008 412 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
Nylon Membrane, positively charged (20×30 cm) (10×15 cm) (0.3×3 m roll)	 10 sheets 20 sheets 1 roll	 11 209 272 001 11 209 299 001 11 417 240 001

* available from Roche Diagnostics

Available Printed Material:

DIG Product Selection Guide	03 189 236 001
DIG Appl. Manual f. Filter Hybridization	11 438 425 001
DIG Appl. Manual for Nonradioactive In Situ Hybridization	11 630 067 001

Changes to previous version

Editorial changes.

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