For life science research only.
Not for use in diagnostic procedures.

DIG-High Prime

Version: 10
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For the nonradioactive labeling of DNA with DIG-11-dUTP, alkali-labile using random oligonucleotides as primers.

Cat. No. 11 585 606 910 160 µl
40 labeling assays

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG-High Prime, 5x conc.	Random prime labeling mixture: Premixed solution of 1 U/µl Klenow polymerase, labeling grade, 1 mM dATP, dCTP, dGTP each, 0.65 mM dTTP, 0.35 mM DIG-11-dUTP, alkali-labile, 5x stabilized reaction buffer in 50% (v/v) glycerol.	1 vial, 160 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	DIG-High Prime, 5x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***To avoid contamination, aliquot and store the solution in 2 to 3 vials.***

1.3. Additional Equipment and Reagent required

For random primed DNA labeling

- 0.2 M EDTA, pH 8.0
- Autoclaved, double-distilled water
- Water bath
- Ice bath

For labeling DNA isolated from agarose

- High Pure PCR Product Purification Kit*

For detection of DIG-labeled DNA

- Anti-Digoxigenin-AP, Fab fragments*
- DIG Nucleic Acid Detection Kit*
- DIG Luminescent Detection Kit*

1.4. Application

DIG-High Prime-labeled probes are used in a variety of hybridization reactions:
- Southern blots
- Northern blots
- Dot/slot blots
- Screening of gene libraries
- *In situ* hybridizations

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

- DNA fragments of at least 100 bp.
- Linearized plasmid, cosmid, or λDNA.
- Supercoiled DNA
- Minimal amounts of DNA (10 ng), such as DNA restriction fragments isolated from gels or in molten agarose.

General Considerations

Template DNA requirements

Feature	Detail
Purity	▪ For plasmid DNA, use the High Pure Plasmid Isolation Kit* for purification. ▪ When other commercially available purification kits are used, perform an additional phenol/chloroform extraction to remove residual protein. ℹ *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 have a size of ≥100 bp. ▪ Template DNA >5 kb should be restriction digested using a 4 bp cutter prior to labeling.
Amount	For the Random primed DNA labeling protocol, 0.01 to 3 µg of template can be labeled. ℹ *Larger amounts can be labeled by scaling up of all components and volumes. 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.*

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Working Solution

Solution	Composition	Use	Storage and Stability
Water	Autoclaved, double-distilled water	Dilution of DNA.	Store at +15 to +25°C.
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0	Stops the reaction.	Store at +15 to +25°C.

2.2. Protocols

Random primed DNA labeling

Perform the standard random primed DNA labeling according to the following steps.

1. To a reaction vial, add 1 µg template DNA (linear or supercoiled) and autoclaved, double-distilled water to a final volume of 16 µl.

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2. Denature the DNA by heating in a boiling water bath for 10 minutes.
   – Quickly chill in an ice water bath.
   Full denaturation is essential for efficient labeling.

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3. Mix DIG-High Prime thoroughly and add 4 µl to the denatured DNA; mix and centrifuge briefly.
   – Incubate for 1 hour or overnight at +37°C.
   Longer incubations up to 20 hours increase the yield of DIG-labeled DNA, see Table, **Labeling reaction yield**.

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4. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0, and/or by heating to +65°C for 10 minutes.

The length of the DIG-labeled fragments obtained with DIG-High Prime ranges from 200 bp to ≥1,000 bp, depending on the length of the original template.

Labeling DNA isolated from agarose

1. For hybridization of genomic Southern blots, separate the template insert DNA from the vector by agarose gel electrophoresis.

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2. Isolate DNA from the gel using the High Pure PCR Product Purification Kit * or an agarose gel DNA extraction kit for DNA fragments in the range of 400 bp to 5 kbp.
   – The kit can be used for standard agarose gels as well as low-melting point agarose gels.

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.*

Labeling reaction yield

The labeling efficiency is shown in the following table and in Figure 1.

Template DNA [ng]	Template DNA [ng] and Labeling Time	
	1 Hour	20 Hours
10	45	600
30	130	1,050
100	270	1,500
300	450	2,000
1,000	850	2,300
3,000	1,350	2,650

Reactions were performed with increasing amounts of different template DNA for 1 hour and 20 hours. The yield of DIG-labeled DNA was determined by incorporation of a radioactive tracer and confirmed by a dot blot. Numbers shown are the average of 10 independent labeling assays.

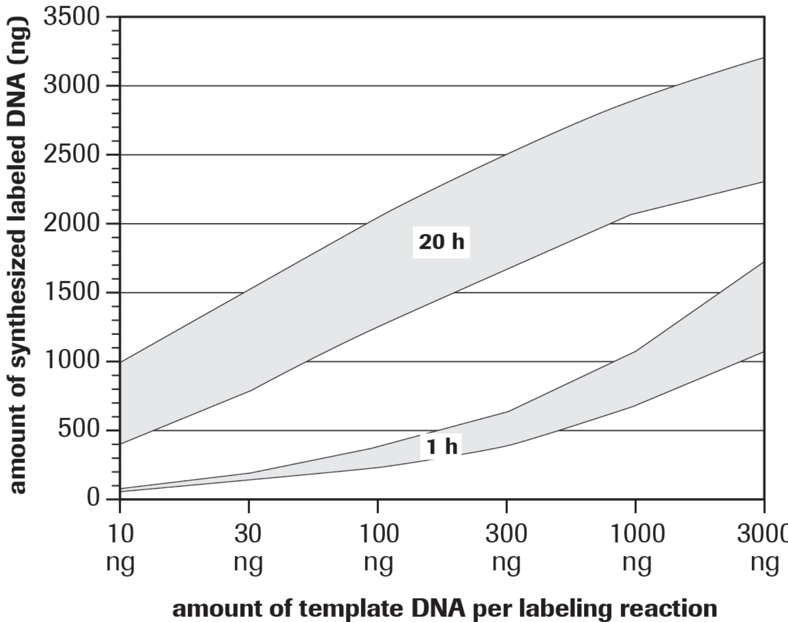

Fig. 1: Yield of DIG-labeled DNA from different amounts of template DNAs for 1 h and 20 h incubation of the DIG-High Prime reaction at +37°C.

Detection of DIG-labeled DNA

Detect DIG-labeled DNA using Anti-DIG-AP, Fab fragments* which catalyze a color or a chemiluminescent reaction. Special kits are available for color detection (DIG Nucleic Acid Detection Kit*) or 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.

Semi-quantitative determination of labeling efficiency

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 step causes background, while too low of a concentration leads to weak signals.
The preferred method for quantification of labeled probes is the direct detection method.

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.

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② The nylon membrane is subjected to immunological detection with Anti-Digoxigenin-AP, Fab fragments* and CSPD, ready-to-use*.
– The intensities of the dilution series of DIG-labeled DNA and control DNA are compared by exposure to an imaging device, X-ray film, or Lumi-Film*.

Probe quantification

To prepare the dilution series shown below, dilute labeled probes and the DIG-labeled Control DNA* to 1 ng/µl, according to the expected yield of synthesized nucleic acid. The expected yield of DIG-labeled DNA in your probe can best be estimated by referring to the Table in, **Labeling reaction yield**. The yield depends on the starting amount of template and incubation time.

ℹ *The yields given in the Table,* **Labeling reaction yield** *were achieved under optimal conditions using highly purified template DNA.*

Dilution series

Prepare a dilution series of your labeled probe and your control DNA as described.

See the Instructions for Use of the DIG-High Prime DNA Labeling and Detection Starter Kit II for the protocol and working solutions.*

Tube	DNA [µl]	From Tube No.	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 from 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, see Table, **Labeling reaction yield**, and can be used in the recommended concentration in the hybridization.

2.3. Parameters

Chemical Name

Structural formula

Fig. 2: Structure of alkali-labile DIG-11-dUTP.

3. Additional Information on this Product

3.1. Test Principle

DIG-labeled DNA probes are generated with DIG-High Prime according to the random primed labeling technique. DIG-High Prime is a specifically developed reaction mixture containing DIG-11-dUTP, and all reagents necessary for random-primed labeling. The premixed DIG-High-Prime reduces pipetting steps and increases yield, reproducibility, and convenience.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Agarose Gel DNA Extraction Kit	1 kit, up to 100 reactions	11 696 505 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
High Pure Plasmid Isolation Kit	1 kit, 50 purifications	11 754 777 001
	1 kit, 250 purifications	11 754 785 001
Lumi-Film Chemiluminescent Detection Film	100 films, 7.1 x 9.4 inches, 18 x 24 cm, *Not available in US*	11 666 916 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
DIG-labeled Control DNA	50 µl, 5 µg/ml DIG-labeled plasmid DNA	11 585 738 910
DIG-High Prime DNA Labeling and Detection Starter Kit II	1 kit, 12 labeling reactions of 10 ng to 3 µg DNA and detection of 24 blots of 100 cm^2	11 585 614 910

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications,
please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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