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

DIG DNA Labeling Kit

Version: 16
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Random primed DNA labeling with digoxigenin-dUTP, alkali-labile

Cat. No. 11 175 033 910 1 kit
40 labeling reactions of 10 ng to 3 µg DNA

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG DNA Labeling Kit, Control-DNA 1 unlabeled	▪ 100 µg/ml in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0. ▪ Mixture of pBR328 DNA digested separately with *Bam* HI, *Bgl* I, and *Hinf* I. The separate digests are combined in a ratio of 2:3:3. ▪ Sizes of the 16 pBR328 fragments: 4,907, 2,176, 1,766, 1,230, 1,033, 653, 517, 453, 394, 298 (2 ×), 234 (2 ×), 220, and 154 (2 ×) bp. ▪ Control target in a Southern blot.	1 vial, 20 µl
2	DIG DNA Labeling Kit, Control-DNA 2 unlabeled	▪ 200 µg/ml ▪ pBR328 DNA that has been linearized with *Bam* HI. ▪ To practice labeling and to check labeling efficiency.	1 vial, 20 µl
3	DIG DNA Labeling Kit, DNA dilution buffer	▪ 50 µg/ml herring sperm DNA in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0 (+20°C). ▪ For the dilution steps in the semi-quantitative determination of the labeling efficiency.	2 vials, 1 ml each
4	DIG DNA Labeling Kit, Control DNA labeled	▪ Linearized pBR328 DNA labeled with digoxigenin according to the standard protocol. ▪ 1 µg template DNA and approximately 250 ng digoxigenin-labeled DNA. ▪ To estimate the yield of DIG-labeled DNA.	1 vial, 50 µl
5	DIG DNA Labeling Kit, Hexanucleotide mixture, 10x conc.	▪ 62.5 A_{260} units/ml random hexanucleotides in 500 mM Tris-HCl, 100 mM $MgCl_2$, 1 mM dithioerythritol (DTE]), 2 mg/ml BSA; pH 7.2. ▪ Component of the labeling reaction.	1 vial, 80 µl
6	DIG DNA Labeling Kit, dNTP-labeling mixture, 10x conc.	▪ 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM DIG-11-dUTP, alkali-labile; pH 7.5 (+20°C). ▪ Component of the labeling reaction.	1 vial, 80 µl
7	DIG DNA Labeling Kit, Klenow enzyme labeling grade	▪ 2 units/µl DNA Polymerase I (Klenow enzyme, large fragment). ▪ Synthesis of DIG-labeled DNA.	1 vial, 40 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Control-DNA 1 unlabeled	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	Control-DNA 2 unlabeled	
3	DNA dilution buffer	
4	Control DNA labeled	
5	Hexanucleotide mixture, 10x conc.	
6	dNTP-labeling mixture, 10x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing. Aliquot and store in 2 to 3 portions.***
7	Klenow enzyme labeling grade	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For Random Primed DNA Labeling

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

For Labeling DNA Isolated from Agarose

- Agarose Gel DNA Extraction Kit*
- High Pure PCR Product Purification Kit*

For Detection of DIG-Labeled DNA

- DIG Nucleic Acid Detection Kit*
- DIG Luminescent Detection Kit*

1.4. Application

DIG-labeled DNA probes can be used in a variety of applications:

- All types of filter hybridization according to our standard protocol in the Instructions for Use of the DIG Easy Hyb* hybridization solution.
- Single-copy gene detection in total genomic DNA, even from organisms with high complexity, such as human, barley, and wheat.
- *In situ* hybridizations.

1.5. Preparation Time

Assay Time

Labeling: One hour to overnight.

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.

General Considerations

Precautions

- Work under sterile conditions.
- Autoclave DIG System solutions.
- Filter-sterilize solutions containing SDS.
- Tween 20 should be added to previously sterilized solutions.
- Rigorously clean and rinse incubation trays before each use.
- Wear powder-free gloves when handling membrane.
- Handle membrane only on the edges and with clean forceps.

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. *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 to 10,000 bp. ▪ Template DNA >10kb should be restriction digested using a 4 bp cutter prior to labeling.
Amount	For the Random Primed DNA Labeling protocol, 10 ng to 3 µg of template can be labeled. *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.*

Working Solution

Solution	Composition	Use	Storage and Stability
Water	Autoclaved, double-distilled water	Dilution of DNA.	+15 to +25°C
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0	Stops the reaction.	+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 10 ng to 3 µg DNA and autoclaved, double-distilled water to a final volume of 15 µl.
   – For a control labeling reaction, use 5 µl of Control-DNA 2 unlabeled (Vial 2) and add 10 µl double-distilled water.

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

3. Add the following to the freshly denatured probe or Control DNA:

Reagent	Volume [µl]
Hexanucleotide mixture, 10x conc. (Vial 5)	2
dNTP-labeling mixture (Vial 6)	2
Klenow enzyme labeling grade (Vial 7)	1

   – Mix and centrifuge briefly.
   – Incubate for 1 to 20 hours (overnight) at +37°C.
   Longer incubation (up to 20 hours) will increase the yield of labeled DNA, **see Table, Labeling Reaction Yield**.

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 range from 200 to 1,000 bp.

Labeling DNA Isolated from Agarose

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

2. Isolate DNA from the gel using the 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.
The amount of newly synthesized DIG-labeled DNA increases with the amount of template DNA in the labeling reaction and the length of incubation time at +37°C.

Template DNA [ng]	Template DNA [ng] and Labeling Time	
	1 Hour	20 Hours
10	15	50
30	30	120
100	60	260
300	120	450
1,000	260	780
3,000	530	890

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.

Detection of DIG-Labeled DNA

DIG-labeled DNA is detected after fixation and hybridization by an antibody conjugated to the enzyme alkaline phosphatase, which catalyzes 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 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 (Vial 4) 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 X-ray film or Lumi-Film*.

Probe Quantification

To prepare the dilution series shown below, labeled probes and the Control DNA labeled (Vial 4) must be diluted 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.

The Control DNA labeled (Vial 4) contains approximately 250 ng DIG-labeled DNA in 50 µl (5 µg/ml). Prepare a 1:5 dilution of the Control DNA as starting material for the dilution series below.

The yields given in the Table, **Labeling Reaction Yield** *were 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:

Tube	DNA [µl]	From Tube No.	DNA Dilution Buffer (Vial 3) [µl]	Dilution	Final Concentration
1	–	Dilution of probe and Vial 4	–	–	1 ng/µl
2	2	1	198	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

Direct Detection

1. Apply 1 µl spots of Tubes 3 to 10 from your labeled probe and the Control DNA labeled to a strip of Nylon Membrane*.

2. Fix the nucleic acid to the membrane by crosslinking with UV-light or baking for 30 minutes at +120°C.

3. Follow the chemiluminescent detection protocol described in the Instructions for Use of the DIG Liminescent Detection Kit* or of the substrates CSPD* or CDP-*Star** using volumes appropriate to the size of your membrane strip.

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 in Labeling Reaction Yield***, and can be used in the recommended concentration in the hybridization.

2.3. Parameters

Sensitivity

A human single-copy gene is detected with a DIG-labeled DNA probe in a Southern blot of 1 µg digested human DNA (see Figure 1).

3. Results

Genomic Southern Blot

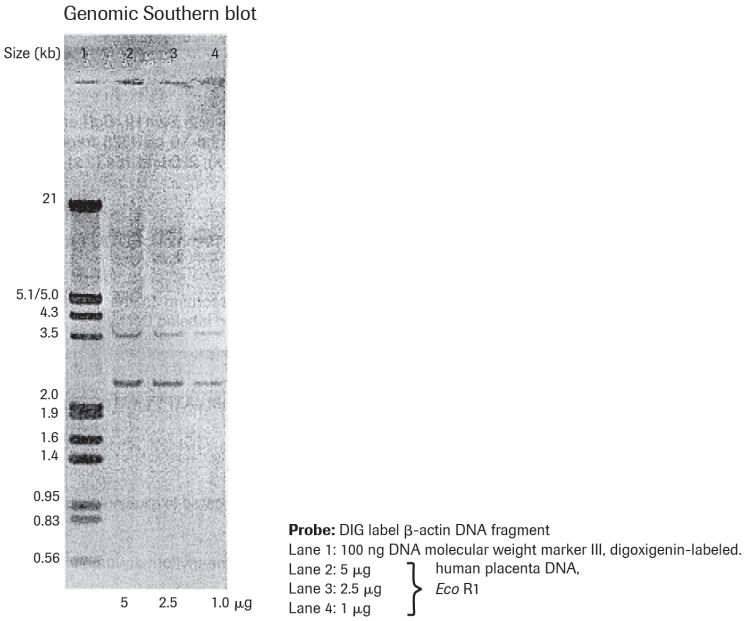

Fig. 1: Detection of a single-copy gene (β-actin) in total human DNA using the standard protocol.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low sensitivity	Inefficient probe labeling.	Check labeling efficiency. The labeling reaction can be upscaled. Prolong incubation time to overnight.
		Clean up template DNA by phenolization.
		Use only fragments <5 kb or predigest with a restriction enzyme, such as a 4 bp cutter.
		Make sure that template is efficiently denatured before labeling.
	Low probe concentration in the hybridization.	Increase probe concentration, but do not use more than 25 ng/ml DNA probe.
		Check hybridization and washing conditions.
		Prolong hybridization time.
High background	Inefficient hybridization	Recalculate hybridization temperature.
		Do not allow the membrane to dry between prehybridization and hybridization.
		If using plastic bags, remove all air bubbles prior to sealing.
	Wrong type of nylon membrane.	Some types of nylon membrane may cause high background. Use Nylon Membranes, positively charged*, especially tested for the Roche DIG System.
	Inefficient blocking before immunoassay.	Prolong blocking and washing steps.
	Ineffective stringency washes.	Check temperature of stringency washes; prewarm wash solution to correct temperature.

5. Additional Information on this Product

5.1. Test Principle

DIG-labeled DNA probes are generated according to the random-primed DNA labeling method which is based on the hybridization of random oligonucleotides to the denatured DNA template. The complementary DNA strand is synthesized by Klenow enzyme which uses the 3′-OH termini of the random oligonucleotides as primers and a mixture of deoxyribonucleotides containing DIG-11-dUTP, alkali-labile for elongation (see Figure 2). This results in incorporation of digoxigenin into the newly synthesized DNA.

The use of the alkali-labile form of DIG-11-dUTP enables easier and more efficient stripping of blots for rehybridization experiments with a second DIG-labeled probe.

DNA probes labeled with DIG-11-dUTP, alkali-labile cannot be denatured using NaOH; instead, denature by boiling in a water bath.

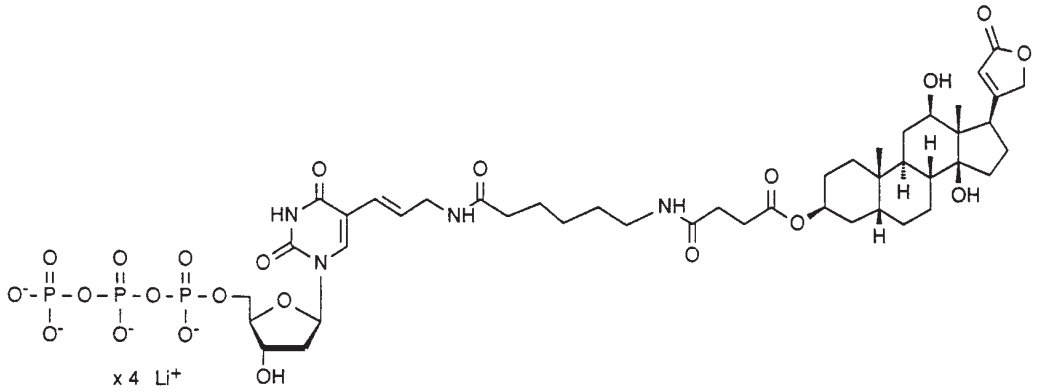

Fig. 2: Chemical structure of DIG-11-dUTP, alkali-labile.

5.2. Quality Control

Using 30 ng/ml of the DIG-labeled control DNA pBR 328 (Vial 4) as a hybridization probe, 0.1 pg homologous DNA diluted into 50 µg heterologous DNA can be detected in a Southern blot after a 16 hour color development or 0.03 pg after <30 minutes exposure to an X-ray film when using the chemiluminescence substrate CSPD.

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
DIG Easy Hyb Granules	6 bottles, Granules for 6 x 100 ml	11 796 895 001
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
High Pure Plasmid Isolation Kit	1 kit, 50 purifications	11 754 777 001
	1 kit, 250 purifications	11 754 785 001
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
Agarose Gel DNA Extraction Kit	1 kit, up to 100 reactions	11 696 505 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
DIG Easy Hyb	500 ml	11 603 558 001
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
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
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910

6.4. Trademarks

HIGH PURE and DIG EASY HYB are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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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