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

DIG DNA Labeling and Detection Kit

Version: 21
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Random primed DNA labeling with digoxigenin-dUTP, alkali-labile and detection of hybrids by enzyme immunoassay.

Cat. No. 11 093 657 910 1 kit
25 labeling reactions of 10 ng - 3 µg DNA and detection of 50 blots of 100 cm^2

Store the kit at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG DNA Labeling and Detection Kit, Control DNA 1 unlabeled	▪ Clear solution ▪ pBR328, 100 µg/ml, digested separately with Bam HI, Bgl I, and Hinf I. ▪ Digests are combined in a ratio of 2:3:3. ▪ 16 pBR328 fragment sizes: 4,907, 2,176, 1,766, 1,230, 1,033, 653, 517, 453, 394, 298 (2 ×), 220, and 154 (2 ×) bp. ▪ Control DNA for Southern blot.	1 vial, 20 µl
2	DIG DNA Labeling and Detection Kit, Control DNA 2 unlabeled	▪ Clear solution ▪ pBR328, 200 µg/ml ▪ Linearized with Bam HI ▪ Control for labeling reaction.	1 vial, 20 µl
3	DIG DNA Labeling and Detection Kit, DNA dilution buffer	▪ Clear solution ▪ 50 µg/ml herring sperm DNA in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0 at +20°C.	2 vials, 1 ml each
4	DIG DNA Labeling and Detection Kit, DIG-labeled control DNA	▪ Clear solution ▪ pBR328 DNA linearized with Bam HI, 5 µg/ml ▪ Determination of the labeling efficiency.	1 vial, 50 µl
5	DIG DNA Labeling and Detection Kit, Hexanucleotide mixture, 10x conc.	▪ Clear solution ▪ Component of the labeling reaction.	1 vial, 50 µl
6	DIG DNA Labeling and Detection Kit, dNTP-labeling mixture, 10x conc.	▪ Clear solution ▪ 1 mM each dATP, dGTP, dCTP, 0.65 mM dTTP, 0.35 mM DIG-11-dUTP, pH 7.5 (+20°C). ▪ Component of the labeling reaction.	1 vial, 50 µl
7	DIG DNA Labeling and Detection Kit, Klenow enzyme	▪ Clear solution, 2 U/µl ▪ Enzyme for the DNA synthesis.	1 vial, 25 µl
8	DIG DNA Labeling and Detection Kit, Anti-DIG-AP conjugate	▪ Clear solution, 750 U/ml ▪ Polyclonal sheep anti-digoxigenin, Fab-fragments, conjugated to alkaline phosphatase.	1 vial, 200 µl
9	DIG DNA Labeling and Detection Kit, NBT/BCIP stock solution	▪ Clear solution, color may vary between light yellow and brown. ▪ Concentrated stock solution. ▪ Reacts with alkaline phosphatase.	10 vials, 1 ml each
10	DIG DNA Labeling and Detection Kit, Blocking Reagent	Powder	2 bottles, 50 g each

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.
2	Control DNA 2 unlabeled	
3	DNA dilution buffer	
4	DIG-labeled control DNA	
5	Hexanucleotide mixture, 10x conc.	
6	dNTP-labeling mixture, 10x conc.	
7	Klenow enzyme	
8	Anti-DIG-AP conjugate	Store at +2 to +8°C.
9	NBT/BCIP stock solution	Store at +2 to +8°C or at least 4 weeks at +15 to +25°C. ⚠ ***During shipment of the kit, a precipitate may occur which is easily dissolved by briefly warming to +37°C.***
10	Blocking Reagent	▪ Store dry at +2 to +8°C or +15 to +25°C. ▪ Store 10x-concentrated solution in aliquots at −15 to −25°C or +2 to +8°C for 1 month under sterile conditions. ⚠ ***Always prepare working solution fresh.***

Storage Conditions (Working Solution)

Additional solutions

Solution	Storage
Water	Store at +15 to +25°C.
EDTA	

1.3. Additional Equipment and Reagent required

For DIG DNA labeling

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

For determination of labeling efficiency

See section, **Working Solution** *for information on preparing solutions.*

- Nylon membranes, positively charged*
- DIG Wash and Block Buffer Set* and
- TE buffer, or
- Washing buffer
- Maleic acid buffer
- Detection buffer
- TE buffer

For DNA transfer and fixation

- UV light box, or
- UV crosslinker
- 2x SSC*, or
- 10x SSC*

For Hybridization

- DIG Easy Hyb*
- Ice/water
- Shaking water bath, or
- Hybridization oven
- Nylon membranes, positively charged*
- Hybridization bags, or
- Temperature resistant plastic or glass boxes, Petri dishes, roller bottles, or sealable plastic bags

⚠ ***Do not use open trays when working with DIG Easy Hyb buffer.***

For Immunological detection

See section, **Working Solution** *for information on preparing solutions.*

- Temperature resistant plastic bags or roller bottles
- Hybridization bags
- DIG Wash and Block Buffer Set*
- TE buffer, or
- Washing buffer
- Maleic acid buffer
- Detection buffer
- TE buffer

For Stripping and reprobing of DNA blots

- Dimethylformamide (DMF)
- 0.2 M NaOH, 0.1% SDS* (w/v)
- 2x SSC*
- Large tray
- Water bath

1.4. Application

DIG-labeled DNA probes can be used for all types of filter hybridization.

1.5. Preparation Time

Assay Time

Step	Reaction Time [hours]
DNA labeling	1 to overnight
Hybridization	6 to overnight
Immunological detection	1.5
Color development	0.5 – 16

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- DNA fragments of at least 100 bp
- Linearized plasmid, cosmid or λDNA
- Supercoiled DNA

General Considerations

Precautions

When performing DIG labeling and detection, follow these guidelines:

Recommendation	Guideline
Work under clean conditions.	▪ Autoclave DIG System solutions. ▪ Filter-sterilize solutions containing SDS. ▪ Tween 20* should be added to previously sterilized solutions.
Use clean incubation trays.	▪ Carefully clean and rinse laboratory trays before each use.
Membrane handling requirements.	▪ Wear powder-free gloves. ▪ Handle membrane only at the edges and with clean forceps.

Template DNA

Parameter	Recommendation
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 or larger. ▪ Template DNA >10 kb should be restriction-digested using a 4 bp cutter, such as Hae III prior to labeling.
Amount	With the procedure described, 10 ng to 3 µg of template can be labeled. ▪ By scaling up of all volumes and components accordingly, this procedure can be used for labeling 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.

Working Solution

Solution	Composition/Preparation	Storage and Stability	For use in...
Washing buffer	0.1 M Maleic acid, 0.15 M NaCl, pH 7.5 (+20°C), 0.3% (v/v) Tween 20*	Store at +15 to +25°C.	Removal of unbound antibody.
Maleic acid buffer	0.1 M Maleic acid, 0.15 M NaCl; adjust with NaOH (solid) to pH 7.5 (+20°C)		Dilution of Blocking solution.
Detection buffer	0.1 M Tris-HCl*, 0.1 M NaCl, pH 9.5 (+20°C)		Adjustment of the pH to 9.5.
TE buffer	10 mM Tris-HCl*, 1 mM EDTA, pH 8.0		Stopping the color reaction.
Blocking stock solution, 10x conc.	Dissolve Blocking Reagent (Bottle 10) 10% (w/v) in Maleic acid buffer under constant stirring on a heating block (+65°C), or heat in a microwave oven, autoclave. *The solution remains opaque.*	Store at +2 to +8°C.	Preparation of blocking solution.
Blocking solution	Prepare a 1x working solution by diluting the 10x Blocking solution 1:10 in Maleic acid buffer.	***Always prepare fresh.***	Blocking of nonspecific binding sites on the membrane.
Antibody solution	▪ Centrifuge Anti-DIG-AP conjugate (Vial 8) for 5 minutes at 10,000 rpm in the original vial prior to each use; pipette the necessary amount carefully from the surface. ▪ Dilute Anti-DIG-AP conjugate 1:5,000 (150 mU/ml) in Blocking solution.	Store 12 hours at +2 to +8°C.	Binding to the DIG-labeled probe.
Color substrate solution	Add 40 µl of NBT/BCIP stock solution (Vial 9) to 2 ml of Detection buffer. ***Keep protected from light.***	***Always prepare fresh.***	Visualization of antibody binding.

Immunological detection

Solution	Composition/Preparation	Storage and Stability	For use in...
Washing buffer	0.1 M Maleic acid, 0.15 M NaCl, pH 7.5 (+20°C), 0.3% (v/v) Tween 20*	Store at +15 to +25°C.	Washing of membrane.
Maleic acid buffer	0.1 M Maleic acid, 0.15 M NaCl; adjust with NaOH (solid) to pH 7.5 (+20°C)		Dilution of Blocking solution.
Detection buffer	0.1 M Tris-HCl*, 0.1 M NaCl, pH 9.5 (+20°C)		As alkaline phosphatase buffer.
TE buffer	10 mM Tris-HCl*, 1 mM EDTA, pH 8.0		Stopping the color reaction.
Blocking stock solution, 10x conc.	Dissolve Blocking Reagent (Bottle 10) 10% (w/v) in Maleic acid buffer under constant stirring on a heating block (+65°C), or heat in a microwave oven, autoclave. *The solution remains opaque.*	Store at +2 to +8°C.	Preparation of blocking solution.
Blocking solution	Prepare a 1x working solution by diluting the 10x Blocking solution 1:10 in Maleic acid buffer.	***Always prepare fresh.***	Blocking of nonspecific binding sites.
Antibody solution	▪ Centrifuge Anti-DIG-AP conjugate (Vial 8) for 5 minutes at 10,000 rpm in the original vial prior to each use; pipette the necessary amount carefully from the surface. ▪ Dilute Anti-DIG-AP conjugate 1:5,000 (150 mU/ml) in Blocking solution.	Store 12 hours at +2 to +8°C.	Binding to the DIG-labeled probe.
Color substrate solution	Add 200 µl of NBT/BCIP stock solution (Vial 9) to 10 ml of Detection buffer. ***Keep protected from light.***	***Always prepare fresh.***	Visualization of antibody binding

2.2. Protocols

DIG DNA labeling

DNA is random primed labeled with Digoxigenin-11-dUTP using a mixture of random hexamers, a dNTP mix containing alkali-labile Digoxigenin-11-dUTP, and labeling grade Klenow enzyme.

Larger amounts can be labeled by scaling up of all components and volumes. Linear DNA is labeled more efficiently than circular and supercoiled DNA.

1. Add 10 ng to 3 µg DNA and autoclaved, double distilled water to a final volume of 15 µl to a reaction vial.
   – For a control labeling reaction, use 5 µl of Control-DNA 2 unlabeled (Vial 2) and add 10 µl double-distilled water.

2. Heat in a boiling water bath for 10 minutes to denature the DNA.
   – Rapidly cool down in an ice/water bath.
   Full denaturation is essential for efficient labeling.

3. Add the following reagents 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 (Vial 7)	1

   – Mix and centrifuge briefly.
   – Incubate for 1 to 20 hours to overnight at +37°C.
   Longer incubation up to 20 hours will increase the yield of labeled DNA, see section, **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 ranges from 200 to 1,000 bp.

Labeling reaction yield

Labeling reactions were performed with increasing amounts of different template DNAs for 1 and 20 hours. The yield of DIG-labeled DNA was determined by incorporation of a radioactive tracer and confirmed by a dot blot (Average of 10 independent labeling assays).

Template DNA [ng]	1 hour [ng]	20 hours [ng]
10	15	50
30	30	120
100	60	260
300	120	500
1,000	260	780
3,000	530	890

Labeling of DNA isolated from agarose

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

2. To isolate DNA from the gel, use a gel DNA extraction kit for DNA fragments or the High Pure PCR Product Purification Kit* in the range of 400 bp to 5 kbp.
   – Kits 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.

3. Purify labeled probes with the High Pure PCR Product Purification Kit* to remove residual agarose particles.

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 mix causes background, while too low of a concentration leads to weak signals. The preferred method for the 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*.

② Part of the nylon membrane is preloaded with defined dilutions of DIG-labeled control DNA which are used as standards.

③ The nylon membrane is then subjected to immunological detection with anti-digoxigenin-AP conjugate and the freshly prepared Color substrate solution.

④ The color intensities of the dilution series of DIG-labeled DNA and control DNA are compared and amounts calculated.

Dilution series

Labeled probes and the DIG-labeled control DNA (Vial 4) must be diluted to 1 ng/µl, according to the expected yield of synthesized nucleic acid, to start the dilution series. The expected yield of DIG-labeled DNA in your probe can best be estimated, see section, **Labeling reaction yield**. The yield depends on the starting amount of template and incubation time.

i The following yields were achieved under optimal conditions with highly purified template DNA.

Tube	DNA [µl]	From tube No.	DNA dilution buffer (Vial 3) [µl]	Dilution	Final conc.
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

Direct detection

i See section, **Working Solution** *for information on preparing solutions.*

i Use sufficient buffer volumes to completely cover the membrane during all steps.

❶ Apply a 1 µl spot from tubes 2 to 9 from your labeled probes and the labeled control to a small strip of nylon membrane.

❷ Fix the nucleic acid to the membrane by cross linking with UV light or baking for 30 minutes at +120°C.

❸ Transfer the membrane into a plastic container with 20 ml Maleic acid buffer.
– Incubate while shaking for 2 minutes at +15 to +25°C.

❹ Incubate for 30 minutes in 10 ml Blocking solution.

❺ Incubate for 30 minutes in 10 ml Antibody solution.

6 Wash 2 × 15 minutes with 10 ml Washing buffer.

7 Equilibrate 2 to 5 minutes in 10 ml Detection buffer.

8 Incubate membrane in 2 ml freshly prepared Color substrate solution in a lightproof container.
⚠ ***Do not shake during color development.***
i The membrane can be exposed to light for short time periods to monitor color development.

9 Wash the membrane for 5 minutes with autoclaved, double-distilled water or TE buffer to stop the reaction when desired spot intensities are achieved.
– Document the results by photocopying the wet filter or by photography.

DNA transfer and fixation

Transfer methods and membranes

All common types of DNA transfer methods are suitable for subsequent DIG hybridization. Gels lacking ethidium bromide are preferred as ethidium can cause uneven background problems. For best results, use gels blotted by capillary transfer with 20x SSC* on nylon membranes, positively charged*.
⚠ ***Alkali transfer, for example, using 0.4 M NaOH is not suitable for the transfer of DIG-labeled molecular weight markers*.***

Fixation

The following methods can be used to fix the DNA to the membrane:

Method	Steps
UV-crosslinking (nylon membrane)	▪ Place the membrane on Whatman 3MM paper soaked with 10x SSC*. ▪ UV crosslink the wet membrane without prior washing. ▪ Rinse the membrane briefly in double-distilled water and air-dry.
Bake at +120°C (nylon membrane)	▪ Wash the membrane briefly in 2x SSC*. ▪ Bake the nylon membrane at +120°C for 30 minutes or according to the manufacturer`s instructions.
Bake at +80°C (nylon membrane)	▪ Wash the membrane briefly in 2x SSC*. ▪ Bake at +80°C for 2 hours under vacuum.

Membrane storage

IF...	THEN...
you want to continue,	use the membrane immediately for prehybridization.
you not want to continue,	store the membrane dry at +2 to +8°C.

Hybridization

Hybridization temperature

Calculate the temperature based on the GC content and percent homology of probe to target according to the following equation using a standard equation for hybridization solutions containing 50% formamide:

Tm = 49.82 + 0.41 (% G + C) – (600/l) [l = length of hybrid in base pairs]
$T_{opt.}$ = Tm – 20 – 25°C

The actual hybridization temperature $T_{opt.}$ for hybridization with DIG Easy Hyb* buffer is 20 to 25°C below the calculated Tm value. $T_{opt.}$ is a stringent hybridization temperature and allows up to 18% mismatches between probe and target. When the degree of homology of probe to template is <80%, lower $T_{opt.}$ accordingly (approximately 1.4°C below Tm per 1% mismatch) and adjust the stringent washing steps accordingly, that is, increase SSC* concentration and lower washing temperature.

Hybridization protocol

⚠ ***Do not use open containers with DIG Easy Hyb buffer.***

1. Pre-heat an appropriate volume of DIG Easy Hyb* buffer (20 ml/100 cm² filter) to the hybridization temperature.
   – Prehybridize filter for 30 minutes with gentle agitation in an appropriate container.
   i *Ensure that membranes move freely, especially when using several membranes in the same prehybridization solution.*

2. Denature approximately 25 ng/ml DIG-labeled DNA probe by boiling for 5 minutes.
   – Cool rapidly in ice/water.
   i *Since the DIG-11-dUTP is alkali-labile, DNA probes cannot be denatured by alkali treatment (NaOH).*

3. Add denatured DIG-labeled DNA probe to the pre-heated DIG Easy Hyb* buffer (3.5 ml/100 cm² membrane).
   – Mix well; avoid foaming since bubbles may lead to background.

4. Pour off prehybridization solution and add probe/hybridization mixture to the membrane.
   – Incubate at least 6 hours to overnight with gentle agitation at the hybridization temperature.

i *DIG Easy Hyb* substrate containing DIG-labeled probe can be stored at –15 to –25°C and be reused several times when freshly denatured at +68°C for 10 minutes before use. Do not boil DIG Easy Hyb* substrate.*

Stringency washes

For most DNA applications, a stringency wash with 0.5x SSC* is sufficient. The correct post washing conditions must be determined empirically for each probe:

- For human genomic DNA, use 0.5x SSC* and +65°C.
- For probes >150 bp and with a high G/C content, wash at +68°C.
- For shorter probes ≤100 bp, lower the wash temperature.

For the post-hybridization washes:

1. Wash 2 × 5 minutes in ample 2x SSC*, 0.1% SDS* at +15 to +25°C under constant agitation.

2. Wash 2 × 15 minutes in 0.5x SSC*, 0.1% SDS* (prewarmed to wash temperature) at +65 to +68°C under constant agitation.

Immunological detection

After hybridization and stringency washes, perform the immunological detection using a 100 cm^2 membrane.

Perform all incubations at +15 to +25°C with agitation. If the membrane is to be reprobed, do not allow the membrane to dry at any time.

See section, **Working Solution** *for information on preparing solutions.*

1. Rinse membrane for 1 to 5 minutes in Washing buffer.

2. Incubate for 30 minutes in 100 ml Blocking solution.

3. Incubate for 30 minutes in 20 ml Antibody solution.

4. Wash 2 × 15 minutes in 100 ml Washing buffer.

5. Equilibrate 2 to 5 minutes in 20 ml Detection buffer.

6. Incubate membrane in 10 ml freshly prepared Color substrate solution in an appropriate container in the dark.
   Do not shake during color development.
   The color precipitate starts to form within a few minutes and the reaction is usually finished after 16 hours. The membrane can be exposed to light for short time periods to monitor color development.

7. Stop the reaction when the desired spot or band intensities are achieved by washing the membrane for 5 minutes with 50 ml of autoclaved, double-distilled water or TE buffer.
   – Document results by photocopying the wet filter or by photography.

Membrane storage

IF...	THEN...
you want to reprobe the membrane,	do not let membrane dry at any time; store in sealed plastic bags. *If you want to maintain the color, store membranes in TE buffer; do not allow the membrane to dry.*
you do not want to reprobe the membrane,	dry the membrane at +15 to +25°C for storage. *Color fades upon drying; to revitalize the color, wet the membrane in TE buffer.*

Stripping and reprobing of DNA blots

The alkali-labile form of DIG-11-dUTP enables easier and more efficient stripping of blots for rehybridization experiments.

When stripping and rehybridization of blots is planned, the membrane should not dry off at any time.

Work under a fume hood.

1. Heat DMF to +50 to +60°C in a large glass beaker in a water bath under a fume hood.
   – Incubate membranes in the heated DMF until the blue color precipitate is removed from the filter.
   DMF is volatile and can be ignited above +67°C.

2. Rinse membrane briefly in double-distilled water.

3. Wash for 2 × 20 minutes in 0.2 M NaOH, SDS* 0.1% (w/v) at +37°C under constant agitation.

4. Equilibrate briefly in 2x SSC*.

5. Air-dry or use directly for hybridization.

2.3. Parameters

Sensitivity

A single copy human gene is detected in a Southern blot of 1 µg digested placenta DNA.

Sensitivity depends both on the concentration of labeled DNA in the hybridization and on the time of the color reaction.

3. Results

Probe	DIG-labeled ß-actin DNA fragment
Template	Lane 1: 100 ng DNA Molecular Weight Marker III, digoxigenin-labeled* Lane 2: 5 µg human placenta DNA, Eco RI Lane 3: 2.5 µg human placenta DNA, Eco RI Lane 4: 1 µg human placenta DNA, Eco RI

Genomic Southern Blot

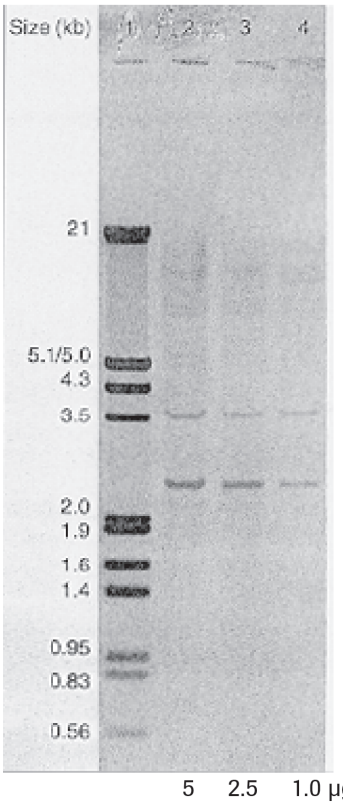

Fig.1: This figure shows the detection of the ß-actin gene in total human DNA using the standard protocol.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low sensitivity obtained.	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 four bp cutter.
		Make sure that template is efficiently denatured before labeling.
	Low probe concentration in the hybridization.	Increase probe concentration; do not use >25 ng/ml DNA probe.
		Check hybridization and washing conditions.
		Prolong hybridization time.
		Prolong color development to 16 hours.
High background present.	Inefficient hybridization	Recalculate hybridization temperature.
		Do not allow the membrane to dry between prehybridization and hybridization.
		Remove all air bubbles prior to sealing plastic bags.
	Wrong type of nylon membrane.	Some types of nylon membrane may cause high background; use nylon membrane*, especially tested for the 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.
	Special hints for immunoassay.	When using laboratory trays for the detection procedure, clean them rigorously before use.
		Perform Anti-DIG-AP binding and color development in separate trays.

5. Additional Information on this Product

5.1. Test Principle

Labeling principle

DIG-labeled DNA probes are generated according to the method of random primed labeling 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. DIG dUTP is incorporated every 20 to 25 nucleotides into the newly synthesized DNA. This density of haptens in the DNA yields the highest sensitivity in the detection reaction.

Test principle

The DIG DNA Labeling and Detection Kit uses digoxigenin (DIG), a steroid hapten to label DNA probes for hybridization and subsequent color detection by enzyme immunoassay.

Stage	Description
DNA labeling	DIG-labeled DNA probes are generated according to the random primed labeling technique.
Hybridization	DIG-labeled probes are used for hybridization to membrane blotted nucleic acids according to standard methods. The use of the alkali-labile form of DIG-11-dUTP enables easier and more efficient stripping of blots for rehybridization with a second DIG-labeled probe.
Immunological detection	The hybridized probes are immunodetected with anti-digoxigenin- AP, Fab fragments and are then visualized with the colorimetric substrates NBT/BCIP.

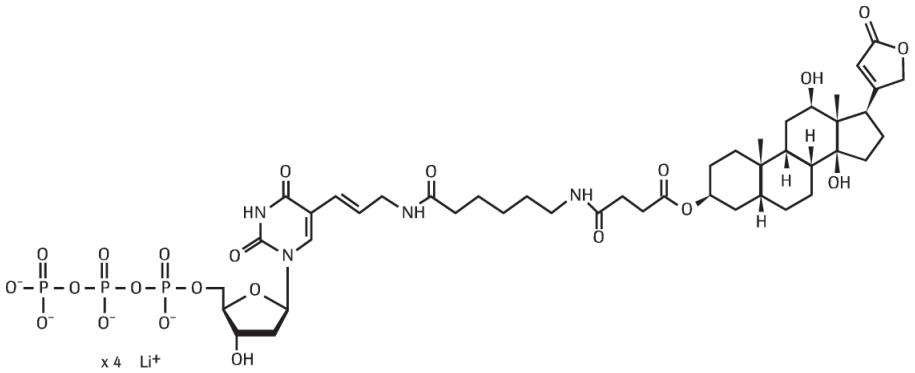

Fig. 2: The figure shows DIG-11-dUTP.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support.**

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		
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
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
DIG Easy Hyb	500 ml	11 603 558 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
High Pure Plasmid Isolation Kit	1 kit, 50 purifications	11 754 777 001
	1 kit, 250 purifications	11 754 785 001
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
DNA Molecular Weight Marker II, DIG-labeled	5 µg, 500 µl	11 218 590 910
DNA Molecular Weight Marker III, DIG-labeled	5 µg, 500 µl	11 218 603 910
DNA Molecular Weight Marker VI, DIG-labeled	5 µg, 500 µl	11 218 611 910
DNA Molecular Weight Marker VII, DIG-labeled	5 µg, 500 µl	11 669 940 910
DNA Molecular Weight Marker VIII, DIG-labeled	5 µg, 500 µl	11 449 451 910
Tris hydrochloride	500 g	10 812 846 001

6.4. Trademarks

DIG EASY HYB is a trademark 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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