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Not for use in diagnostic procedures.

DIG Nucleic Acid Detection Kit

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Detection of digoxigenin-labeled nucleic acids by enzyme immunoassay and enzyme-catalyzed color reaction with NBT/BCIP.

Cat. No. 11 175 041 910 1 kit
Detection of 40 blots of 10 cm x 10 cm

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	DIG Nucleic Acid Detection Kit, Control-DNA labeled	▪ 5 µg/mL pBR328 DNA (linearized with Bam HI). ▪ Clear solution. ▪ For the determination of labeling efficiency.	1 vial, 50 µL
2	DIG Nucleic Acid Detection Kit, DNA dilution buffer	▪ 50 µg/mL fish sperm DNA in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0 at +25°C. ▪ Clear solution.	1 vial, 1 mL
3	DIG Nucleic Acid Detection Kit, Anti-DIG AP-conjugate	▪ 750 U/mL, polyclonal sheep anti-digoxigenin, Fab-fragments, conjugated to alkaline phosphatase. ▪ Clear solution.	1 vial, 200 µL
4	DIG Nucleic Acid Detection Kit, NBT/BCIP stock solution, 50x conc.	▪ 18.75 mg/mL nitroblue tetrazolium chloride and 9.4 mg/mL 5-bromo-4-chloro-3-indolyl-phosphate, toluidine salt in 67% (v/v) DMSO. ▪ Color varies between light yellow and brown clear solution, depending on the lot. ⚠ ***The color does not impair the quality or function of the substrate.*** ▪ Precipitating chromogenic substrate for alkaline phosphatase.	8 vials, 1 mL each
5	DIG Nucleic Acid 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 expiration date printed on the label.

Vial / bottle	Label	Storage
1	Control-DNA labeled	Store at −15 to −25°C.
2	DNA dilution buffer	
3	Anti-DIG AP-conjugate	Store at −15 to −25°C. After opening, store at +2 to +8°C.
4	NBT/BCIP stock solution, 50x conc.	Store at −15 to −25°C. After opening, store at +2 to +8°C. ⚠ ***Keep protected from light.*** ⚠ ***During shipment of the kit on dry ice, a precipitate may occur which is easily dissolved by briefly warming to +37°C.***
5	Blocking reagent	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Immunological detection

See section, **Working Solution** *for additional information on how to prepare solutions.*

- Container of appropriate size in relation to filter size
- Shaking water bath
- Photocopy device or camera
- TE buffer or double-distilled water
- Washing buffer
- Maleic acid buffer
- Detection buffer

The Washing buffer, Maleic acid buffer, and Detection buffer are available in the DIG Wash and Block Buffer Set, DNase and RNase free.*

Stripping and reprobing of DNA blots

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

1.4. Application

Use the DIG Nucleic Acid Detection Kit for a variety of applications:

- Southern and northern blots
- Other nucleic acid blotting applications
- *In situ* hybridization

1.5. Preparation Time

Assay Time

Step	Reaction time [Hours]
Immunological detection	1.5
Color development	0.5 to 16

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The DIG Nucleic Acid Detection Kit is used with DIG-labeled nucleic acids.

Working Solution

Immunological detection

The Washing buffer, Maleic acid buffer, and Detection buffer are also available in the DIG Wash and Block Buffer Set DNase and RNase free.*

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 solid NaOH 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 pH 9.5.

Preparation of kit working solutions

The Blocking solution is also available in the DIG Wash and Block Buffer Set DNase and RNase free.*

Solution	Composition / preparation	Storage and stability	For use in
Blocking reagent, 10x conc.	Dissolve Blocking reagent (Bottle 5) in Maleic acid buffer to a final concentration of 10% (w/v) with shaking and heating either on a heating block or in a microwave oven. ⚠ ***Autoclave stock solution.***	Store at +2 to +8°C or −15 to −25°C.	Preparation of Blocking solution.
Blocking solution, 1x conc.	Dilute 10x Blocking reagent 1:10 with Maleic acid buffer.	⚠ ***Always prepare fresh.***	Blocking of nonspecific binding sites.
Antibody solution	▪ Centrifuge Anti-Digoxigenin-AP (Vial 3) for 5 minutes at 10,000 rpm in the original vial prior to each use, and pipette the necessary amount carefully from the surface. ▪ Dilute Anti-Digoxigenin-AP 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 4) to 10 mL of Detection buffer.	⚠ ***Always prepare fresh.*** ⚠ ***Keep protected from light.***	Visualization of antibody binding.

2.2. Protocols

Immunological detection

See section, **Working Solution** *for additional information on preparation of solutions.*
The following procedure describes the immunological detection for a 10 cm × 10 cm blot.

1. After hybridization and stringency washes, rinse membrane briefly 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 and incubate 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 in approximately 16 hours. The membrane can be exposed to light for short time periods to monitor color development.

7. Stop the reaction with 50 mL of sterile, double-distilled water or with TE buffer when desired spot or band intensities are obtained.
   – Document the results by photocopying the wet filter or by photography.

Membrane storage

IF...	THEN...
you want to reprobe the membrane,	store membrane in sealed plastic bags containing TE buffer. The color remains unchanged. ⚠ ***Do not let membranes dry out if they are to be reprobed.***
you do not want to reprobe the membrane,	dry the membrane at +15 to +25°C and store. *Color fades upon drying but can be revitalized by wetting the membrane with TE buffer.*

Stripping and reprobing of membranes

When stripping and rehybridization is planned, do not let the membrane dry out.
⚠ ***Always work under a fume hood.***

1. Heat a large beaker in a water bath with DMF to +50 to +60°C.
   – Incubate the membranes in the heated DMF until the blue color precipitate is removed from the filter.

   ⚠ ***DMF is volatile and can be ignited at >+67°C.***

2. Rinse membrane briefly in double-distilled water.

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

4. Equilibrate briefly in 2x SSC.

5. Air dry membrane or use directly for hybridization.

2.3. Parameters

Sensitivity

The gene for tissue plasminogen activator (tPA) is detected in a Southern blot in 1 µg digested human placenta DNA.

3. Results

DNA hybridization

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

Concentration of labeled DNA [ng/mL]	0.5	2	5	10	20	30	50
pg homologous DNA detectable after a color reaction of:							
1 hour	–	10	5	2	1	1	0.5
3 hours	10	2	1	0.5	0.5	0.5	0.2
16 hours	2	0.5	0.2	0.1	0.1	0.05	0.05

RNA hybridization

Sensitivity depends both on the concentration of labeled RNA in the hybridization solution and on the duration of color reaction.

Concentration of labeled RNA [ng/mL]	2	5	10	20	50	100	200
pg homologous RNA detectable after a color reaction of:							
1 hour	–	10	3	3	1	0.3	0.3
3 hours	10	3	1	0.3	0.3	0.1	0.1
16 hours	3	1	0.3	0.1	0.1	0.03	0.03

4. Troubleshooting

Observation	Possible cause	Recommendation
Low sensitivity observed.	Inefficient probe labeling.	Check labeling efficiency; the labeling reaction can be upscaled, or incubation time prolonged 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 the template is efficiently denatured before labeling.
	Low probe concentration in the hybridization.	Increase probe concentration, but do not use >25 ng/mL DNA probe or >100 ng/mL RNA 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.
		If you use plastic bags, remove all air bubbles prior to sealing.
	Wrong type of membrane.	Some types of nylon membrane may cause high background; use Nylon Membranes*, 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, rigorously clean them before use. ⚠ ***Always perform Anti-DIG-AP binding and color development in separate trays.***

5. Additional Information on this Product

5.1. Test Principle

DIG-labeled DNA, RNA, or oligonucleotide probes are detected after hybridization to target nucleic acids by enzyme-linked immunoassay using an antibody conjugate, such as anti-digoxigenin alkaline phosphatase conjugate (anti-DIG-AP). A subsequent enzyme-catalyzed color reaction with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium salt (NBT) produces an insoluble blue precipitate which visualizes hybrid molecules.

Colorimetric reaction

BCIP is the AP substrate which reacts further after the dephosphorylation to give a dark-blue indigo dye as an oxidation product. NBT serves herein as the oxidant and gives also a dark-blue dye. It intensifies the color and makes the detection more sensitive.

Immunological detection

Hybridized filters can be detected immediately after stringent washings or stored dry for later detection. After blocking of the membrane, binding of anti-DIG-AP to hybridized DIG-labeled nucleic acids occurs. The color reaction is initiated at alkaline pH by the addition of BCIP and NBT. A blue precipitate starts to form within a few minutes and continues up to three days. Typically, the reaction can be terminated when the color precipitate is clearly visible, which can vary from 1 to 24 hours. Background is usually not observed due to the use of highly specific antibody Fab fragments and an efficient blocking step.

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

Editorial changes.
The section, **Preparation of kit working solutions** has been missing and has now been added again.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
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
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
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

6.4. Trademarks

All 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:
Product Disclaimers.

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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