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

DIG Luminescent Detection Kit

Version: 09
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Chemiluminescent detection of digoxigenin-labeled nucleic acids by enzyme immunoassay.

Cat. No. 11 363 514 910 1 kit
50 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 Luminescent Detection Kit, Control DNA labeled	▪ Linearized pBR328 DNA, labeled with digoxigenin according to the standard protocol, containing 1 µg template DNA and approximately 260 ng synthesized labeled DNA. ▪ Clear solution. ▪ For estimation of labeling efficiency.	1 vial, 50 µl
2	DIG Luminescent Detection Kit, DNA dilution buffer	▪ 50 µg/ml fish sperm DNA in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0 at +20°C. ▪ Clear solution.	1 vial, 1 ml
3	DIG Luminescent Detection Kit, Anti-DIG-AP	▪ Polyclonal sheep, Fab fragments from an anti-digoxigenin antibody, conjugated with alkaline phosphatase (AP). ▪ Clear solution.	1 vial, 100 µl
4	DIG Luminescent Detection Kit, Blocking reagent	Powder	2 bottles, 50 g each
5	DIG Luminescent Detection Kit, CSPD	▪ Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro)tricyclo [3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate ▪ Molecular weight: 461 Da ▪ Chemiluminescent substrate for alkaline phosphatase.	1 bottle, 1 ml

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 labeled	Store at −15 to −25°C.
2	DNA dilution buffer	
3	Anti-DIG-AP	Store at +2 to +8°C after opening.
4	Blocking reagent	Store dry at +2 to +8°C or +15 to +25°C after opening.
5	CSPD	Store at +2 to +8°C if used frequently. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

For immunological detection

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

- Hybridization bags or
- Development folders
- Plastic or glass boxes or petri dishes
- DIG Wash and Block Buffer Set* or
- Washing buffer
- Maleic acid buffer
- Detection buffer

For stripping and reprobing of DNA blots

- Large beaker
- Water bath
- Water, double-distilled
- 10x SSC*
- 10% SDS*
- 0.2 M NaOH

1.4. Application

The DIG Luminescent Detection Kit is used for highly sensitive detection of DIG-labeled nucleic acids on all types of membrane blots, using anti-digoxigenin, alkaline phosphatase conjugates and the chemiluminescent substrate CSPD.

1.5. Preparation Time

Assay Time

Overview

Step	Time
Immunological detection	1.5 hours
Signal detection	5 to 30 minutes

Immunological detection with CSPD

Step	Time [min]
Washing and blocking of membrane	32
Antibody binding	30
Washing and equilibration of membrane	32
Luminescent reaction	5
Preincubation at +37°C	10
Film exposure	20
Total time	**130**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The DIG Luminescent Kit is used with DIG-labeled nucleic acids.

General Considerations

DIG-labeling reaction

Labeling techniques for DNA, RNA, and oligonucleotides are shown in the following table.

Nucleic Acid	Labeling Reaction
DNA probes	Labeling with Digoxigenin-11-dUTP* via random-primed labeling, nick translation, or PCR.
Oligonucleotides	▪ 3′-end labeling using Digoxigenin-11-ddUTP* or tailed with Digoxigenin-11-dUTP* using Terminal transferase*. ▪ 5′-end labeling of oligonucleotides using DIG-NHS-ester* (Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic-acid-N-hydroxysuccinimide ester).
RNA probes	Synthesis via *in vitro* transcription reaction using SP6, T7, or T3 RNA Polymerases*.

Working Solution

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

Immunological detection with CSPD			
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 to 9.5.
Preparation of kit working solutions			
CSPD	Dilute CSPD 1:100 in Detection buffer. *The solution can be reused one to two times.*	Store at +2 to +8°C. ***Keep protected from light.***	Chemiluminescence detection.
Antibody solution	▪ Centrifuge Anti-DIG-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, Fab fragments 1:10,000 (75 mU/ml) in Blocking solution.	Store at +2 to +8°C for 12 hours.	Binding to the DIG-labeled probe.

Blocking stock solution, 10x conc.	Dissolve Blocking Reagent 10% (w/v) in Maleic acid buffer under constant stirring on a heating block (+65°C), or heat in a microwave oven and autoclave. *The solution remains opaque*	Store at +2 to +8°C initially; after first usage, store in aliquots at −15 to −25°C.	Preparation of Blocking solution.
Blocking solution, 1x	Dilute the 10x Blocking solution 1:10 in Maleic acid buffer.	***Always prepare fresh.***	Blocking of nonspecific binding sites on the membrane.

2.2. Protocols

Immunological detection with CSPD

The following steps describe how to perform the immunological detection on a 100 cm^2 (10 cm × 10 cm) membrane.
Perform all incubations at +20 to +25°C with agitation.

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. Place membrane with DNA side facing up on a development folder or hybridization bag and apply 2 ml CSPD working solution.
   – Immediately cover the membrane with the second sheet of the folder to spread the substrate evenly and without air bubbles over the membrane.
   – Incubate for 5 minutes.
7. Squeeze out excess liquid and seal the edges of the development folder.
8. Incubate the damp membrane for 5 to 15 minutes at +37°C to enhance the luminescent reaction.
9. Expose to an imaging instrument, or to X-ray film or Lumi-Film* for 5 to 25 minutes at +15 to +25°C.
   Luminescence continues for at least 24 hours and signal intensity remains almost constant during the first hours. Multiple exposures can be taken to achieve the desired signal strength, see section, **Results, Figure 1**.

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.
If filters are to be stripped and reprobed, they should not be allowed to dry out, and should be stored in 2x SSC or Maleic acid buffer.

1. Rinse membrane thoroughly in double-distilled water.
2. Wash for 2 × 15 minutes at +37°C in 0.2 M NaOH containing 0.1% SDS* to remove the DIG-labeled probe.
3. Rinse thoroughly 5 minutes in 2x SSC*.
4. Prehybridize and hybridize with a second probe.

2.3. Parameters

Sensitivity

The gene for tissue plasminogen activator (tPA) is detected in a Southern blot in 0.3 µg restriction enzyme-digested human placenta DNA. Using DIG-labeled RNA probes, similar sensitivity is obtained.

3. Results

Detection of DIG-labeled nucleic acids with CSPD

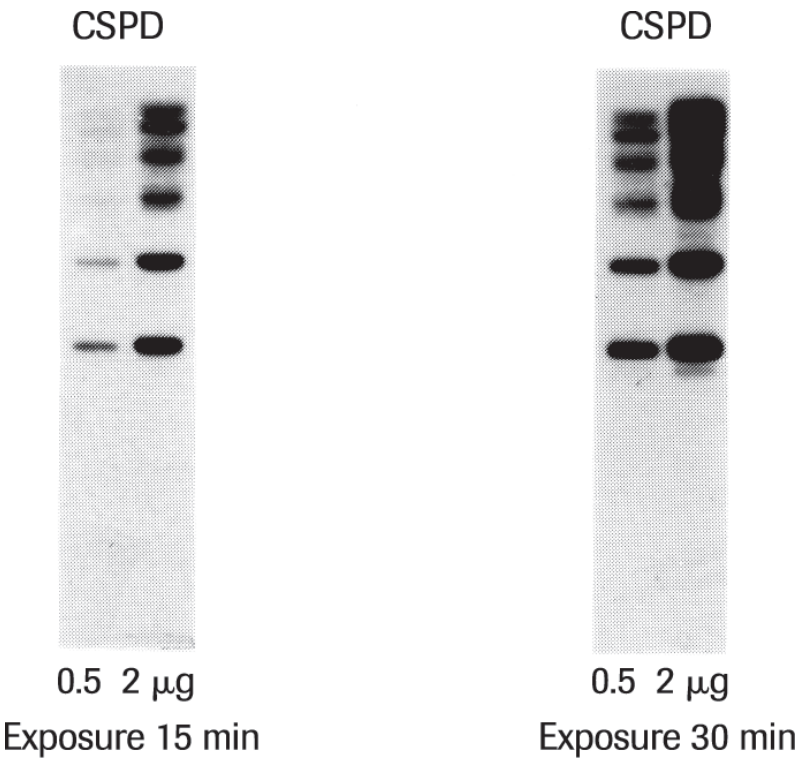

Fig. 1: Human genomic DNA digested with Eco RI, separated on a 1% agarose gel, and blotted onto positively charged nylon membranes. The blots were hybridized with 50 ng/ml DIG-labeled β-actin RNA. Chemiluminescent detection performed according to the standard DIG chemiluminescent detection procedure using CSPD at a final concentration of 0.25 mM.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low sensitivity observed.	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 <10 kb or predigest with a restriction enzyme, such as a four bp cutter.
		Check the amount and quality of target DNA.
		Make sure that the template is efficiently denatured before labeling.
	Low probe concentration in the hybridization.	Increase probe concentration; use 25 ng/ml.
		Prolong hybridization time to overnight.
		Increase concentration of Anti-DIG-AP; dilute 1:5,000.
High background present	Inefficient hybridization conditions.	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.
		Use DIG Easy Hyb* buffer, especially when other membrane brands are used.
	Concentration of labeled probe too high.	Determine optimal probe concentration, see section, **Immunological detection with CSPD**; do not use more than 25 ng/ml. ▪ Decrease probe concentration. ▪ Increase volume of prehybridization solution.
	Wrong type of nylon membrane.	Some types of nylon membranes can cause background problems. Use Nylon Membranes, positively charged* especially tested for the DIG system.
	Inefficient blocking before immunoassay.	Prolong blocking and washing steps.
		When using laboratory trays for the detection procedure, rigorously clean trays before use. Perform Anti-DIG-AP binding and chemiluminescent development in separate trays.
		Perform a heat treatment of all glassware to minimize background.

5. Additional Information on this Product

5.1. Test Principle

The nonradioactive DIG system uses digoxigenin, a steroid hapten, coupled to dUTP, UTP, or ddUTP to label DNA, RNA, or oligonucleotides for hybridization and subsequent luminescent detection (Fig. 2).

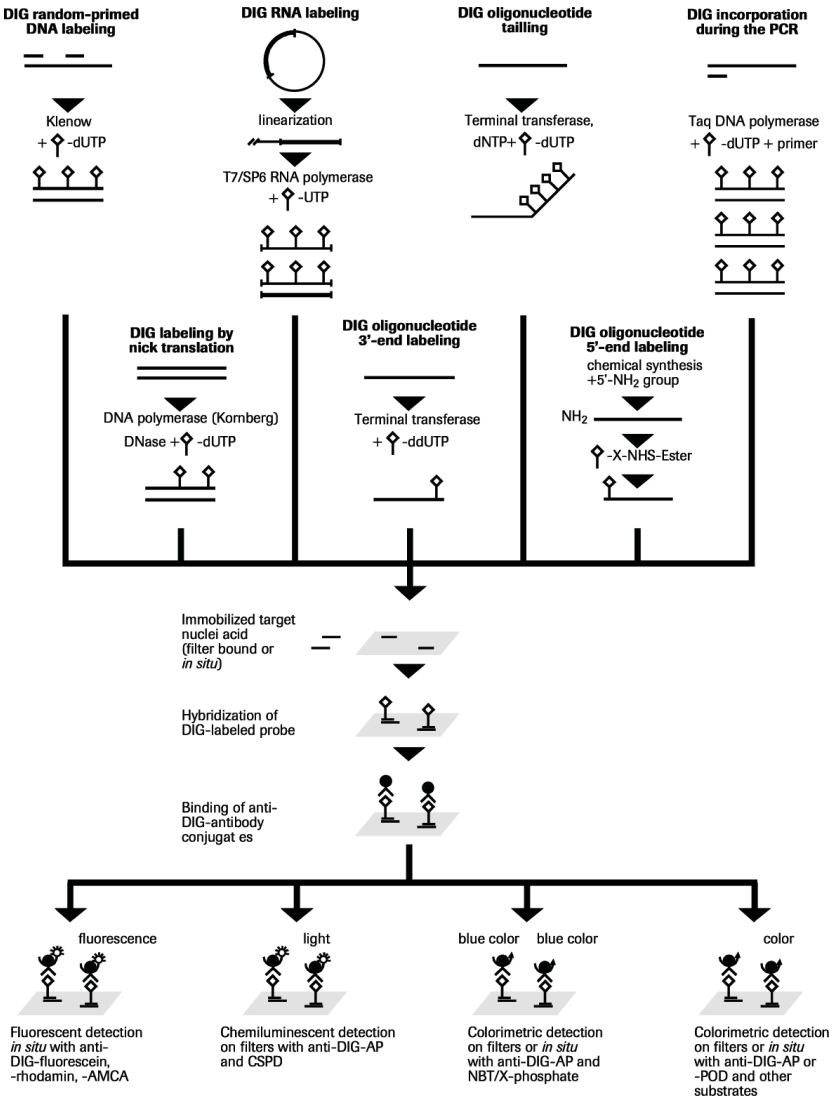

Fig. 2

Immunological detection

① The hybridized probes are immunodetected with anti-digoxigenin, Fab fragments conjugated to alkaline phosphatase and visualized with the chemiluminescence substrate CSPD (Fig. 3).

② Enzymatic dephosphorylation of CSPD by alkaline phosphatase leads to a light emission at a maximum wavelength of 477 nm which is recorded on X-ray film or Lumi-Film*.
– The chemiluminescent signal from CSPD persist for days on nylon membranes.

③ Since film exposures of a few minutes are usually sufficient for signal detection, multiple images may be acquired.

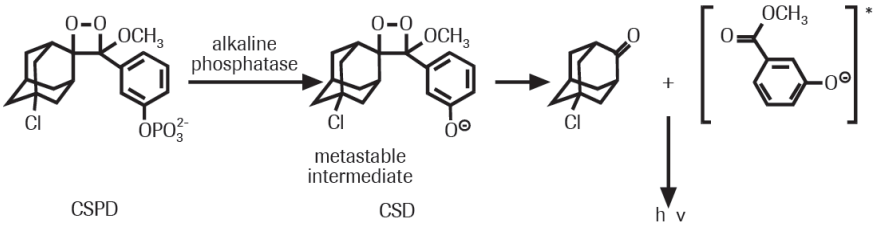

Fig. 3

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		
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 Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Digoxigenin-11-ddUTP	25 nmol, 25 µl, 1 mM	11 363 905 910
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910
Digoxigenin-11-UTP	250 nmol, 25 µl, 10 mM	11 209 256 910
	200 nmol, 57 µl, 3.5 mM	03 359 247 910
Terminal Transferase	8,000 U, 400 U/µl, 20 tailing or 3'-end labeling reactions (400 U per reaction)	03 333 566 001
	24,000 U, 400 U/µl, 60 tailing or 3'-end labeling reactions (400 U per reaction)	03 333 574 001
Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester	5 mg	11 333 054 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Tris hydrochloride	500 g	10 812 846 001
DIG RNA Labeling Kit (SP6/T7)	1 kit, 2 x 10 labeling reactions	11 175 025 910
T3 RNA Polymerase	1,000 U, ≥ 20 U/µl	11 031 163 001
	5,000 U, ≥ 20 U/µl	11 031 171 001

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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