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

Roche

CSPD, ready-to-use

Disodium 3-(4-methoxyspiro {l,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.$1^{3,7}$]decan}-4-yl) phenyl phosphate

Cat. No. 11 755 633 001 2 × 50 ml

Version 07
Content version: March 2016
Store at +2 to +8°C

1. What this Product Does

Contents

- 0.25 mM solution (0.116 mg/ml), 1× conc.
- 2 bottles with dropper, 50 ml each

Storage and Stability

Store the unopened bottle at +2 to +8°C until the expiration date printed on the label.

⚠ Store protected from light.

Application

CSPD is a chemiluminescent substrate for alkaline phosphatase that enables extremely sensitive and fast detection of biomolecules by producing visible light which is recorded with film or instrumentation (1). CSPD can be used for the detection of alkaline phosphatase and alkaline phosphatase conjugates either in solution or on solid supports.

It is especially suited for highly sensitive and fast detection of non-radioactively labeled nucleic acids in

- Southern blots
- Northern blots
- dot blots
- colony or plaque hybridizations
- gel shift assays
- sequencing

⚠ For chemiluminescent detection of nucleic acids with CSPD, the use of nylon membranes is strongly recommended. Nitrocellulose membranes require addition of an enhancer (*e.g.*, Nitroblock, Tropix) to achieve similar signal intensity.

Product Characteristics

Reaction Principle

Enzymatic dephosphorylation of CSPD by alkaline phosphatase leads to the metastable phenolate anion which decomposes and emits light at a maximum wavelength of 477 nm. The luminescent light emission is recorded on X-ray films or by suitable cameras or instruments.

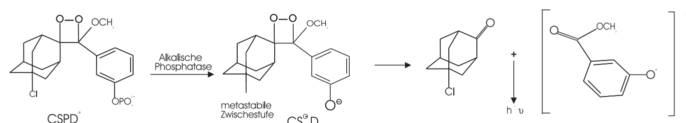

Fig. 1: Reaction scheme

Molecular weight	461
Formula	$C_{18}H_{20}ClO_7PNa_2$
Appearance	clear, colorless solution
Purity	CSPD (purified by HPLC) >98%
Sensitivity	A single-copy gene (tissue plasminogen activator, tPA) is detected in a Southern blot in 0.3 µg *Bgl* II or *Eco* RI digested human placenta DNA. Using DIG-labeled RNA-probes, similar sensitivity is obtained.
Signal stability	The chemiluminescent signal from CSPD persists for days on nylon membranes. Since film exposures of a few minutes are usually sufficient, multiple images may be acquired.

Assay Time

Step	Time
Washing and blocking of membrane	30 min
Antibody binding	30 min
Washing and equilibration of membrane	30 min
Luminescent reaction	5 min
Preincubation at 37°C	10 min
Film exposure	20 min
Total time	**125 min**

2. How to Use this Product

2.1 Before You Begin

Precaution

⚠ Avoid contact and handle with care. Wear gloves and a laboratory coat.

Detection of DIG-labeled Nucleic Acids with CSPD

Nucleic acid probes can be labeled very efficiently with digoxigenin (DIG) and be used as hybridization probes in various membrane blot applications. After stringency washes, the blots are subjected to immunological detection using anti-digoxigenin antibody conjugated to alkaline phosphatase and CSPD. Detailed protocols for DIG labeling and hybridization are available in the product descriptions of various DIG labeling and detection kits (see below) and the DIG Application Manual for Filter Hybridization.

2.2 Detection Procedure for Blot Application

Additional Equipment Required

- hybridization bags*

OR

- temperature resistant plastic or glass boxes, petri dishes or roller bottles

Additional Reagents Required

- Anti-digoxigenin-AP, Fab fragments*
- DIG Wash and Block Buffer Set*

OR

- Washing buffer
- Maleic acid buffer
- Detection buffer

Preparation of Additional Solutions Required

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

Solution	Composition/Preparation	Storage/ Stability	Use
Washing buffer	0.1 M Maleic acid, 0.15 M NaCl; pH 7.5 (+15 to +25°C); 0.3% (v/v) Tween 20	+15 to +25°C, stable	Removal of unbound antibody
Maleic acid buffer	0.1 M Maleic acid, 0.15 M NaCl; adjust with NaOH (solid) to pH 7.5 (+15 to +25°C)	+15 to +25°C, stable	Dilution of Blocking solution
Detection buffer	0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5 (+15 to +25°C)	+15 to +25°C, stable	Adjustment of pH to 9.5
Blocking stock solution, 10×	Dissolve Blocking reagent 10% (w/v) in Maleic acid buffer under constantly stirring on a heating block (+65°C), or heat in a microwave oven, autoclave. The solution remains opaque.	+2 to +8°C	Preparation of Blocking solution
Blocking solution	Prepare a 1× working solution by diluting the 10× Blocking solution 1:10 in Maleic acid buffer.	always prepare fresh	Blocking of unspecific binding sites on the membrane
Antibody solution	Centrifuge the antibody for 5 min at 10,000 rpm in the original vial prior to each use, and pipet the necessary amount carefully from the surface. Dilute anti-digoxigenin-AP 1:10 000 (75 mU/ml) in Blocking solution.	+2 to +8°C	Binding to the DIG-labeled probe

Procedure

The volumes are calculated for a membrane size of 100 cm^2. All incubations should be performed at +15 to +25°C with agitation

Step	Action
1	After hybridization and stringency washes, rinse membrane briefly (1–5 min) in **Washing buffer**
2	Incubate for 30 min in 100 ml **Blocking solution**.
3	Incubate for 30 min in 20 ml **Antibody solution.**
4	Wash 2 × 15 min in 100 ml **Washing buffer**.
5	Equilibrate 2–5 min in 20 ml **Detection buffer**.
6	• Place membrane with DNA side facing up on a development folder (or hybridization bag) and apply 1 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 min at +15 to +25°C.
7	Squeeze out excess liquid and seal the development folder completely. ⚠ Drying of the membrane during exposure will result in dark background.
8	Incubate the damp membrane for 10 min at +37°C to enhance the luminescent reaction.
9	Expose to a luminescent imager for 5–20 min or to X-ray film for 15–25 min at +15 to +25°C. Luminescence continues for at least 48 hours. The signal increases in the first few hours after initiation of the detection reaction until it will reach a plateau where signal intensity remains almost constant during the next 24–48 hours. Multiple exposures can be taken to achieve the desired signal strength.

2.3 Stripping and Reprobing of DNA Blots

General

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

Southern blots hybridized with DIG-labeled RNA probes can be stripped following the same procedure.

Additional Equipment and Reagents Required

- Large tray
- Water bath
- 10× SSC
- 10% SDS
- 0.2 N NaOH

Procedure

This procedure describes the stripping of a membrane.

Alternative stripping protocols, as mentioned in the "DIG Application Manual for Filter Hybridization" (www.roche-applied-science.com/techresources), can also be used with high efficiency.

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

3. Troubleshooting

Problem	Possible Cause	Recommendation
Low sensitivity	Inefficient probe labeling	Check labeling efficiency of your DIG DNA or RNA labeling by comparison to the labeled control DNA or RNA.
	Wrong type of membrane	The quality of the membrane used as support for dot, Southern or northern blotting influences sensitivity and speed of detection. We recommend the nylon membrane, positively charged, from Roche Applied Science, specially tested for chemiluminescent detection. Other types of nylon membranes, for example, Biodyne A (Pall) are also suitable but need longer exposure times to X-ray film. Some membranes may cause strong background formation. Nitrocellulose membranes cannot be used with the protocol described.
	Inefficient hybridization	Increase the concentration of DIG-labeled probe, but do not exceed 25 ng/ml for DNA probes and 100 ng/ml for RNA probes in the hybridization solution. Check hybridization and washing conditions
	Low antibody concentration	Make sure, that the recommended dilution of 1:10 000 was used..
	Exposure time too short	• Increase time of exposure to X-ray film • The type of film may also influence the observed signal strength.
High background	Inefficient labeling	• Purify DNA/RNA by phenol/chloroform extraction and/or ethanol precipitation before labeling • Make sure that the probe does not contain cross-hybridizing vector sequences.
	Wrong type of membrane	• Although the protocol is optimized for the use of positively charged nylon membranes, some types which are very highly charged can cause background. • Lot-to-lot variations in some membranes may also cause problems. When using the recommended nylon membrane* from Roche Applied Science which is function tested with the DIG system, these problems are avoided.
	Concentration of labeled probe too high	It can be necessary to decrease concentration of DIG-labeled DNA or RNA probe. Standard probe concentration for a DNA probe is 25 ng/ml, - for an RNA probe, 100 ng/ml. The critical probe concentration limit (concerning background formation) can be determined by a mock hybridization with increasing probe concentrations using unloaded membrane. • Care should be taken not to permit the membranes to dry throughout the whole procedure.
	Exposure too long	Shorten exposure time. The signal intensity increases with time.

4. Additional Information on this Product

Quality Control

0.03 pg DIG-labeled control-DNA (pBR328/*Bam* HI) diluted with 50 ng heterologous DNA are detected in a dot blot with CSPD after $<$30 min exposure to X-ray film, following the standard detection protocol.

References

1 Bronstein, I., *et al.* (1991) Novel chemiluminescent adamantyl 1,2-dioxetane enzyme substrates in: Stanley P. and Kricka L. J. (eds.), Bioluminescence and Chemiluminescence, Current Status, pp 73-82, John Wiley, Chichester England

5. Supplementary Information

5.1 Conventions

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.
*	Available from Roche Diagnostics

5.2 Ordering Information

Product	Pack Size	Cat No.
DIG Luminescent Det. Kit	1 kit	11 363 514 910
DIG High Prime Labeling and Detection Starter Kit II	12 labeling reactions and 24 blots	11 585 614 910
DIG DNA Labeling and Detection Kit	12 labeling reactions and 24 blots	11 093 657 910
DIG DNA Labeling Kit	40 labeling reactions	11 175 033 910
DIG High Prime	160 µl	11 585 606 910
DIG Easy Hyb Granules	1 set (6 × 100 ml)	11 796 895 001
DIG Easy Hyb	500 ml	11 603 558 001
Blocking Reagent	50 g	11 096 176 001
CSPD	1 ml 2 × 1 ml 4 × 1 ml	11 655 884 001 11 759 035 001 11 759 043 001
Hybridization Bags	50 bags	11 666 649 001
Lumi-Film, for chemiluminescent detection	100 sheets, 20.3 × 24.4 cm 100 sheets, 18 × 24 cm	11 666 657 001 11 666 916 001
Nylon Membrane, positively charged	10 sheets, 20 × 30 cm 20 sheets, 10 × 15 cm 1 roll, 0.3 × 3 m#	11 209 272 001 11 209 299 001 11 417 240 001
Tween 20	2 × 10 ml	11 332 465 001

not available in the US

Changes to Previous Version

Editorial changes

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