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

CSPD

Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate

Version: 13
Content Version: June 2021

Cat. No. 11 655 884 001 1 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	CSPD, Chemiluminescence substrate, 100x conc.	25 mM (11.6 mg/ml) clear, colorless solution.	1 bottle, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	CSPD, 100x conc.	Store at +2 to +8°C. ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

For immunological detection for blot applications

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

- Hybridization Bags, or
  – Temperature-resistant plastic or glass boxes, petri dishes or roller bottles
- Anti-Digoxigenin-AP, Fab fragments*
- Blocking Reagent*
- DIG Wash and Block Buffer Set*, or
  – Washing buffer, Maleic acid buffer, and Detection buffer
- Nylon Membranes, positively charged*
- Luminescent imager, X-ray film, or Lumi-Film*
- Tween 20*
- Tris-HCl*

For stripping and reprobing of DNA blots

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

- Double-distilled water
- Large tray
- Water bath
- 20x SSC*
- 10% SDS*
- 0.2 M NaOH

1.4. 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 on X-ray film or Lumi-Film*, or instrumentation. 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 nonradioactively labeled nucleic acids in the following applications:

- Southern blots
- Northern blots
- Dot blots
- Colony or plaque hybridizations
- Gel shift assays
- Sequencing

⚠ ***For chemiluminescent detection of nucleic acids with CSPD, use nylon membranes. Nitrocellulose membranes require addition of an enhancer, such as Nitro-Block to achieve similar signal intensity.***

1.5. Preparation Time

Assay Time

Step	Time [minutes]
Washing of membrane	1 to 5
Blocking of membrane	30
Antibody binding	30
Washing and equilibration of membrane	30
Luminescent reaction	5
Preincubation at +37°C	10
Film exposure	20
Total time	**125**

2. How to Use this Product

2.1. Before you Begin

Safety Information

Precautions

- Avoid contact with CSPD solution and handle with care.
- Wear gloves and a laboratory coat at all times.

Working Solution

Solution	Composition/Preparation	Storage and Stability	For use in...
CSPD, 1x conc.	Dilute CSPD, 100x conc. 1:100 in Detection buffer (0.1 M Tris-HCl*, 0.1 M NaCl, pH 9.5 at +15 to +25°C).	Store short-term at +2 to +8°C. ⚠ ***Keep protected from light.*** ⚠ ***Can be reused one to two times when kept sterile.***	Substrate for alkaline phosphatase.
Washing buffer	0.1 M maleic acid, 0.15 M NaCl, pH 7.5 (+15 to +25°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 (+15 to +25°C)		Dilution of Blocking solution.
Detection buffer	0.1 M Tris-HCl*, 0.1 M NaCl, pH 9.5 (+15 to +25°C)		Adjustment of pH to 9.5.
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 conc.	Dilute the 10x Blocking solution 1:10 in Maleic acid buffer.	⚠ ***Always prepare fresh.***	Blocking of nonspecific binding sites on the membrane.
Antibody solution	▪ Centrifuge the antibody 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.

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

2.2. Protocols

Immunological detection for blot applications

The following steps describe how to perform the immunological detection on 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.***

1. After hybridization and stringency washes, rinse membrane briefly 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. 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 minutes at +15 to + 25°C.

7. Squeeze out excess liquid and seal the edges of the development folder.
   ⚠ ***Drying of the membrane during exposure will result in dark background.***

8. Incubate the damp membrane for 10 minutes at +37°C to enhance the luminescent reaction.

9. Expose using an imaging instrument for 5 to 20 minutes, or to X-ray film or Lumi-Film* for 15 to 25 minutes at +15 to +25°C.
   ℹ *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 to 48 hours. Multiple exposures can be taken to achieve the desired signal strength.*

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.

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

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

Chemical Formula

$C_{18}H_{20}ClO_7PNa_2$

Molecular Weight

461 Da

Purity

CSPD (HPLC) >98%.

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. Troubleshooting

Observation	Possible cause	Recommendation
Low sensitivity observed.	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.	Use Nylon Membranes, positively charged*, specially tested for chemiluminescent detection. *The quality of the membrane used as support for dot, Southern, or northern blotting influences sensitivity and speed of detection.*
		Other types of nylon membranes are also suitable but need longer exposure times to X-ray or Lumi-Film. *Some membranes may cause strong background formation. Nitrocellulose membranes cannot be used with the protocol described.*
	Inefficient hybridization conditions.	Check hybridization and washing conditions.
		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.
	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 or Lumi-Film.
		The type of film may also influence the observed signal strength.
High background present.	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.	Always use Nylon Membranes, positively charged*, specially tested for chemiluminescent detection. *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. Avoid this by using Nylon Membranes* which are function tested with the DIG system.
	Concentration of labeled probe too high.	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. *Determine the critical probe concentration limit by performing a mock hybridization with increasing probe concentrations using an unloaded membrane.*
		Do not allow the membranes to dry at any time in the procedure.
	Exposure time too long.	Shorten exposure time. *The signal intensity increases with time.*

4. Additional Information on this Product

4.1. Test Principle

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 film or Lumi-Film*, or by suitable cameras or instruments.

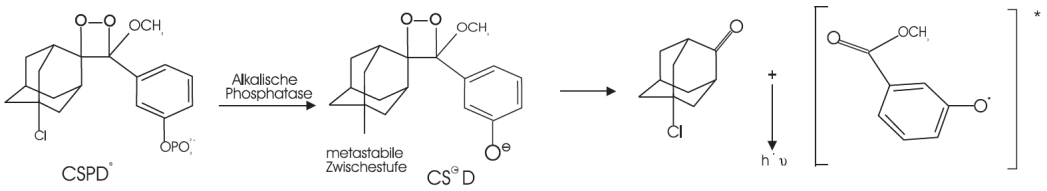

Fig. 1: Reaction principle

4.2. Quality Control

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

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tris hydrochloride	500 g	10 812 846 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
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
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
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Blocking Reagent	50 g	11 096 176 001
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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.

0621.11654004001 ⓑ

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany