CDP-*Star*, ready-to-use

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

Ultra-sensitive and fast chemiluminescent substrate for alkaline phosphatase

Cat. No. 12 041 677 001 2 × 50 ml

Version September 2007

Store in dark at +2 to +8°C

1. What this Product Does

Contents

- 0.25 mM solution (0.124 mg/ml), 1× conc.
- 2 bottles with dropper, 50 ml each

Storage and Stability

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

⚠ Store protected from light.

Application

CDP-*Star* is a chemiluminescent substrate for alkaline phosphatase that enables extremely sensitive and fast detection of biomolecules by producing visible light. Light emission is recorded on X-ray films, with suitable cameras, or on luminescence imager systems (1). CDP-*Star* 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:

- Southern blots
- Northern blots
- dot blots, *e.g.,* cDNA arrays
- colony or plaque hybridizations
- gel shift assays

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

Product Characteristics

Reaction Principle

Enzymatic dephosphorylation of dioxetane by alkaline phosphatase leads to the formation of the meta-stable dioxetane phenolate anion which decomposes and emits light at 466 nm. On nylon membranes, the maximum light emission from CDP-*Star*, ready-to-use, is reached within a few minutes.

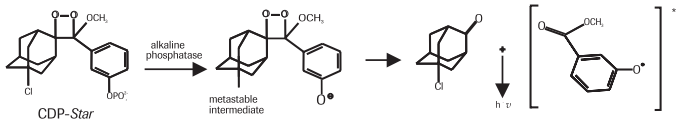

Fig. 1: Reaction scheme

Molecular weight	495.2
Formula	$C_{18}H_{19}Cl_2O_7PNa_2$
Appearance	clear, colorless solution
Purity	CDP-*Star*, ready-to use (purified by HPLC) >98%
Sensitivity	A single-copy gene (tissue plasminogen activator, tPA) is detected in a Southern blot in 3 µg human placental DNA using a DIG-labeled DNA probe and CDP-*Star*, ready-to use, with a film exposure time of < 5 min. Using DIG-labeled RNA probes, similar sensitivity is obtained.
Signal stability	The chemiluminescent signal from CDP-*Star*, ready-to-use, persists for days on nylon membranes. Since film exposures of a few minutes are usually sufficient, multiple images may be acquired.
Advantage	Immediately after substrate addition, CDP-*Star*, ready-to-use, generates a luminescent signal of an approx. 10-fold increased sensitivity, compared to other chemiluminescent substrates, *e.g.,* CSPD*, both on nylon membrane and in solution.

Assay Time

Step	Time
Blocking of membrane	2 min
Washing of membrane	30 min
Antibody binding	30 min
Washing of membrane	2 × 15 min
Equilibration	2 min
Luminescent reactions	5 min
Film exposure	1 min
Total time	**100 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

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 followed by CDP-*Star*, ready-to-use. 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 Applications

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

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 for the substrate reaction
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 and store at +2 to +8°C. The solution remains opaque.	+15 to +25°C	
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	Dilute anti-digoxigenin-AP to 37.5 mU/ml = 1:20 000 in Blocking solution. 🔍 For a 10 cm² blot 20 ml are necessary.	+2 to +8°C	Binding to the DIG-labeled probe

Procedure

The volumes are calculated for a membrane size of 100 cm². All incubations should be performed at +15 to +25°C with agitation.

❶	After hybridization and stringency washes, rinse membrane briefly (1–5 min) in **Washing buffer**.
❷	Incubate for 30 min in 100 ml **Blocking solution**.
❸	Incubate for 30 min in 20 ml **Antibody solution.**
❹	Wash 2 × 15 min in 100 ml **Washing buffer**.
❺	Equilibrate 2–5 min in 20 ml **Detection buffer**.
❻	• Place membrane with DNA side facing up on a development folder (or hybridization bag) and apply 20 drops (0.5 ml) **CDP-*Star*, ready-to-use** solution out of the dropper bottle. • **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.
❼	Squeeze out excess liquid and seal the development folder completely.
❽	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.

2.3 Stripping and Reprobing of DNA Blots

General

If DNA probes are labeled with DIG-11-dUTP, alkali-labile, blots can be stripped efficiently and reprobed, provided that the membranes never dried to completion during the entire procedure. 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

Please refer to the following table.

🔍 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.

❶	Rinse membrane thoroughly in double-distilled water.
❷	Wash for 2 × 15 min at 37°C in 0.2 M NaOH containing 0.1% SDS to remove the DIG-labeled probe.
❸	Rinse thoroughly 5 min in 2× SSC.
❹	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.
	Membrane	The membrane quality 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 can only be used with the protocol described above.
	Hybridization	• Increase the concentration of DIG-labeled DNA or RNA probe in the hybridization solution, but only to a concentration where background is still low. • Reduce the stringency of the washing steps.
	Exposure time	Increase film exposure time. The type of film may also influence the sensitivity. For best results use the Lumi-Film*. Other X-ray films like Cronex 4 (DuPont) or Kodak XAR were tested and can also be recommended.
High background	Labeling	• Purify DNA/RNA by phenol/chloroform extraction and/or ethanol precipitation before labeling. • Make sure that the probe is specific for the target sequence and does not contain cross hybridizing vector sequences.
	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.
	Hybridization	• Important: Especially when using CDP-*Star*, ready-to-use it is of utmost importance to reduce the concentration of the DIG-labeled probe to a minimum (10-20 ng/ml DIG-labeled DNA, 20−50 ng/ml DIG-labeled RNA). In general, half the concentration of labeled probe can be used compared to other detection substrates. The critical probe concentration limit (concerning background) can be determined by hybridizing with increasing probe concentrations to unloaded membranes or homologous dot blots. • Never let the membrane dry out throughout the whole procedure.
	Detection	Decrease concentration of the anti-DIG-AP conjugate to 1: 50,000 (15 mU/ml) in Blocking solution. This does not lead to any significant reduction of sensitivity with CDP-*Star,* ready-to-use as substrate. Increase volumes of the washing and blocking solution and duration of the washing and blocking steps. Spotty background may be caused by precipitates in the anti-DIG-AP conjugate: remove by a short centrifugation step.
	Exposure	Shorten exposure time. The signal intensity increases with time. CDP-*Star* is an extremely fast and sensitive chemiluminescent substrate, which usually requires exposure times of only 15-60 s. The concentration of the CDP-Star, ready-to use, can be reduced with the dropper.

4. Additional Information on this Product

Quality Control

0.03 pg DIG-labeled control DNA (pBR328/*Bam* HI) diluted in 50 ng heterologous DNA are detected in a dot blot with CDP-*Star* after <10 min exposure to X-ray film, following the standard detection protocol.

References

1 Edwards, B. *et al.* (1994). New chemilurninescent 1,2 dioxetane substrates, in: Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects, A.K. Carnpbell, L. Kricka & P.E. Stanley (eds), J. Wiley & Sons, Ltd., Chichester, UK, pp. 56-59

2 Bronstein, I. *et al.* (1989). Chemiluminescent 1,2-dioxetane substrates and their application in the detection of DNA. *Photochem. Photobiol.* **49**, 9

3 Höltke, H.J. *et al.* (1995). The digoxigenin (DIG) system for non-radioactive labeling and detection of nucleic acids - an overview. *Cell. Mol. Biol.* **41**, 883-905

5. Supplementary Information

5.1 Conventions

Symbols

In this package insert 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 Applied Science

5.2 Changes to previous version

- Editorial corrections
- New Quality Control procedure

5.3 Trademarks

5.4 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection: http://www.roche-applied-science.com/dig

Product	Pack Size	Cat No.
DIG Luminescent Detection 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 Ki	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 (ready-to-use hybridization solution without formamide)	500 ml	11 603 558 001
DIG Wash and Block Buffer Set	30 blots (10×10 cm^2)	11 585 762 001
Blocking Reagent	50 g	11 096 176 001
CSPD	1 ml	11 655 884 001
CSPD, ready-to-use	2 × 50 ml	11 755 633 001
CDP-*Star*, ready-to-use	2 × 50 ml	12 041 677 001
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*

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.

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