



Product Information

CDP-*Star*TM Chemiluminescent Substrate Solution

Product No. **C 0712**

Store at 2-8°C

CAS #: [160081-62-9]

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

Product Description

CDP-*Star*TM is a sensitive, chemiluminescent substrate for alkaline phosphatase that allows for the rapid, reproducible detection of alkaline phosphatase-labeled molecules. CDP-*Star* is supplied as a 0.25 mM ready-to-use aqueous solution (i.e. no dilution is necessary) for use in a variety of membrane-based applications, including Northern, Southern, and colony lift blots. CDP-*Star* functions on both neutrally-charged and positively-charged nylon, giving the reagent added application versatility.

Precautions and Disclaimer

CDP-*Star* is for laboratory use only. Not for drug, household or other uses. As the toxicity of this substance has not yet been fully determined, handle with care. A lab coat and gloves should be worn when handling the reagent.

Preparation Instructions

Allow CDP-*Star* to equilibrate to room temperature prior to use. This inhibits formation of condensation within the container that may dilute the substrate. Aseptic technique should be used when transferring CDP-*Star* from the storage container to the membrane.

Storage/Stability

Store CDP-*Star* at 2-8°C when not in use. CDP-*Star* is stable for at least one year when stored under the recommended conditions and handled in an aseptic manner.

Procedure

The procedure below details the processing of a membrane following the completion of a hybridization protocol, including the blocking of non-specific sites on the membrane, incubation with an alkaline phosphatase conjugate, and the final membrane washes.

1. Using forceps, drain off any excess wash buffer and transfer the membrane to a clean, appropriately sized container.
2. Using aseptic technique, add CDP-*Star* to the membrane (50 $\mu\text{l}/\text{cm}^2$ membrane).
3. Incubate for 5 minutes at room temperature with gentle agitation to allow CDP-*Star* to fully cover the membrane.
4. Using forceps, remove the membrane and drain off any excess CDP-*Star* onto an absorbent material. Do not let the membrane dry out.
5. Transfer the membrane to a development folder, with the sample side up. Place into a light-tight film cassette.
6. Expose the membrane to film from 30 seconds to overnight at room temperature. Exposure time should be adjusted accordingly to achieve the highest signal-to-noise ratio.
7. Develop film as per manufacturer's instructions.

Results

Maximum light emission occurs at approximately 60 minutes and continues for up to 24 hours (see Figure 1), thus allowing for multiple film exposures and/or the sensitive detection of targets present in small amounts. The sensitivity of detection depends on probe type, amount of target present, blocking and detection conditions, etc. and should be optimized empirically. While the sensitivity of chemiluminescent detection meets or exceeds that of radioactive detection, it may not exhibit a linear response (see Figure 2).

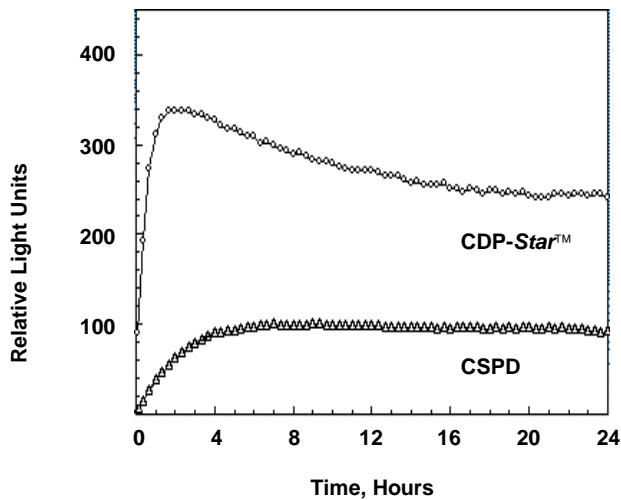


Figure 1. Light Emission Kinetics of CDP-Star and CSPD on Nylon Membrane (Courtesy Tropix, Inc.)

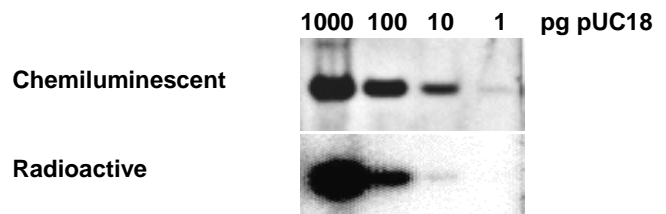


Figure 2. Comparison of Chemiluminescent and Radioactive Detection Methods.

1000, 100, 10, and 1 pg of unlabeled pUC18 plasmid DNA, immobilized on Biobond Plus™ nylon membrane, were hybridized overnight in PerfectHyb Plus™ with a fluorescein (20 ng/ml) or ^{32}P (3×10^8 dpm/ μg)-random primed pUC18 probe, for chemiluminescent and radioactive detection respectively. The membranes were washed under stringent conditions and imaged on an Instant Imager (radioactive) or blocked, incubated with an anti-fluorescein alkaline phosphatase conjugate, washed, and fluorescein-labeled probes detected with CDP-Star (chemiluminescent).

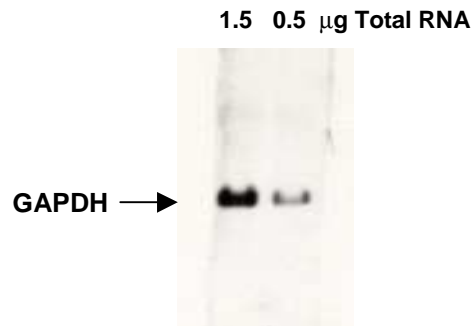


Figure 3. Northern Analysis of HEK293 cells.

1.5 and 0.5 μg total RNA was resolved on a 1.25%, 1X MOPS gel and transferred to BiobondPlus™ nylon membrane. The membrane was hybridized overnight with a fluorescein-labeled GAPDH anti-sense riboprobe (100 ng/ml) in PerfectHyb Plus™. The membrane was blocked, probed, incubated with an anti-fluorescein alkaline phosphatase conjugate, washed, and detected with CDP-Star.

Results

TroubleshootingGuide

Problem	Cause	Solution
Low Signal	Poor transfer efficiency	Poor transfer of nucleic acid to the solid support will decrease the sensitivity. Stain the gel with ethidium bromide prior to hybridization to ensure complete transfer.
	Poor labeling or integrity of probe	Check the efficiency of probe labeling and/or integrity of the probe by comparison to control molecule.
	Insufficient probe concentration	Too low of a concentration of probe may decrease sensitivity. Increase the probe concentration to a level at which background is still low.
	Excessive stringency	Excessive stringency due to too high of temperature and/or too low salt concentration may lead to a decreased sensitivity. Decrease the stringency of the washing steps.
	Blocking conditions	Too much blocking agent may mask the signal. Decrease the amount of blocking agent in blocking buffer.
	Antibody or streptavidin conjugate concentration	Increase the antibody or streptavidin conjugate concentration to allow sensitive detection without background.
	Exposure time	Increase the exposure time.
High Background	Probe purity	Ensure that probe has been purified away from unincorporated label.
	Probe concentration	Excess probe may present non-specific binding issues. Decrease the probe concentration to a level that allows sensitive detection.
	Inefficient blocking	<ul style="list-style-type: none"> • Change blocking reagent. • Increase the concentration of blocking reagent. • Increase the duration of the blocking step.
	Inefficient wash steps	Increase the volume of wash solution, time of wash, and number of the wash steps.
	Antibody or streptavidin conjugate concentration	Decrease the antibody or streptavidin conjugate concentration to allow sensitive detection without background.
	Exposure time	Decrease the exposure time

Related Products

CDP-*Star*[™] is a trademark of Tropix, Inc. Bedford, MA, USA and covered under US Patent 5,326,882 and 4,931,569.

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Product Number	Product Name
N1406, N4031, N3656, N3781, N3906	Biobond [™] Nylon Membrane
N5281, N5781, N5406, N5531, N5656	Biobond-Plus [™] Nylon Membrane
RPF-12-DU	BioProbe Random Primed DNA Labeling Kit-FITC
RPB-11-DU RPB-16-DU RPB-11-DC RPB-7-DA	BioProbe Random Primed DNA Labeling Kit-Biotin
OLB-16-DDU OLF-12-DDU	BioProbe 3'-Oligonucleotide Labeling Kits
RLB-11-U RLB-16-U RLB-11-C RLB-7-A RLF-12-U	BioProbe RNA Transcript Labeling Kits
H7033	PerfectHyb Plus [™] Hybridization Buffer
S6639	20X SSC
L4522	10% Lauryl Sulfate
S2890	Streptavidin-Alkaline Phosphatase conjugate
A4843	Anti-Fluorescein Isothiocyanate Alkaline Phosphatase conjugate
A1054	Anti-Digoxin Alkaline Phosphatase conjugate
F1653, F3912, F4037, F1403, F2540, F2665, F1528, F2028, F1778, F1903	Kodak, Biomax MR Film
E9260, E9385, E9510, E9635	Exposure cassette, stainless steel

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