CSPD™ and CDP-Star™ Substrates

Catalog Numbers T2040, T2098, T2042, T2044, T2043, T2138, T2141, T2142, T2143, T2217, T2210, T2212, T2304, T2305, T2306, T2307, T2308, T2309, T2310, T2145, T2146, T2147, T2218, T2214, and T2216

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Invitrogen™ CSPD™ and CDP-Star™ Substrates are chemiluminescent substrates for alkaline phosphatase (AP) in applications including ELISA, microplate-based nucleic acid detection, western blotting, and southern blotting.

CSPD™ and CDP-Star™ substrates generate a luminescent signal through the dephosphorylation of substrates by AP, producing decomposing anions that emit light. Both substrates provide high sensitivity, rapid X-ray film exposure, superior band resolution, and efficient glow light emission kinetics, allowing for multiple film exposures and use with luminometers without automatic reagent injectors. With CSPD™ substrate, peak light emission is obtained in 10–20 minutes in solution assays, or in approximately 4 hours on a nylon membrane; CDP-Star™ substrate exhibits solution kinetics similar to CSPD™ substrate but reaches peak light emission on a membrane in only 1–2 hours.

CSPD™ and CDP-Star™ substrates require an alkaline hydrophobic environment for efficient light emission, which can be achieved using luminescence enhancers or nylon membranes. These enhancers enable the chemical decomposition of the substrates, resulting in fast reaction kinetics and bright chemiluminescent signals. Luminescence enhancers are designed for use in solution assays or membrane blotting and can be used to adjust the output wavelength of light emission, optimizing signal intensity based on the specific chemiluminescence detection instrumentation.

Chemical information

Product	Molecular weight	CAS No.	Chemical name
CSPD™ Substrate	461 g/mol	142849-53-4	Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.13,7]decan}-4-yl)phenyl phosphate
CDP-Star™ Substrate	496 g/mol	160081-62-9	Disodium 2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo[3.3.1.13,7]decan}-4-yl)-1-phenyl phosphate

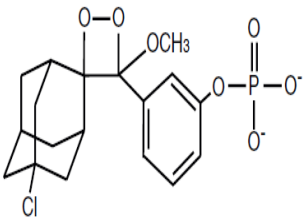

Figure 1 CSPD™ Substrate

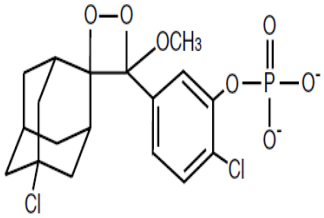

Figure 2 CDP-Star™ Substrate

Contents and storage

Catalog numbers that appear as links open the web pages for those products.

Item	Cat. No.	Amount	Storage
CSPD™ Substrate (25 mM Concentrate)	T2040	0.5 mL	Store at 4°C.[1]
	T2098	1 mL	
	T2042	5 mL	
	T2044	25 mL	
	T2043	10 mL	
	T2138	100 mL	
CSPD™ Substrate (0.25 mM Ready-To-Use)	T2141	50 mL	
	T2142	100 mL	
	T2143	250 mL	
CSPD™ Substrate (0.25 mM Ready-To-Use) with Nitro-Block™ Enhancer	T2217	100 mL	
CSPD™ Substrate (0.4 mM Ready-To-Use) with Sapphire-II™ Enhancer	T2210	100 mL	
CSPD™ Substrate (0.4 mM Ready-To-Use) with Emerald-II™ Enhancer	T2212	100 mL	
CDP-Star™ Substrate (12.5 mM Concentrate)	T2304	1 mL	
	T2305	2 mL	
	T2306	5 mL	
	T2307	10 mL	
	T2308	20 mL	
	T2309	50 mL	
	T2310	200 mL	
CDP-Star™ Substrate (0.25 mM Ready-to-Use)	T2145	50 mL	
	T2146	100 mL	
	T2147	250 mL	
CDP-Star™ Substrate (0.25 mM Ready-to-Use) with Nitro-Block™ Enhancer	T2218	100 mL	
CDP-Star™ Substrate (0.4 mM Ready-to-Use) with Sapphire-II™ Enhancer	T2214	100 mL	
CDP-Star™ Substrate (0.4 mM Ready-to-Use) with Emerald-II™ Enhancer	T2216	100 mL	

[1] The product is stable for at least 1 year when stored as directed.

Required materials not supplied

- Dilution Buffer (0.1 M Diethanolamine containing 1 mM $MgCl_2$)
- 10X Wash Buffer (1X PBS or TBS with 1% Tween 20)
- 10X Blocking Buffer (1X PBS with 0.2% casein)
- 10X Assay Buffer
- Deionized water

Guidelines for selecting a substrate

CSPD™ and CDP-Star™ Substrates are both very sensitive for detection of AP activity, however, CDP-Star™ substrate produces a more intense (brighter) signal than does CSPD™ substrate in either solution or blotting assays. In addition, compared with CSPD™ substrate in a membrane-based assay, CDP-Star™ substrate exhibits a faster time to peak light emission.

- CSPD™ substrate is suitable as a detection reagent for most purposes.
- CDP-Star™ substrate's brighter signal and faster kinetics allow for shorter X-ray film exposure times, making it an ideal for digital image acquisition.
- **Note:** CDP-Star™ substrate may be preferable if maximum signal intensity is needed, shorter film exposure times are desired, or if digital imaging of membrane blots is required.

Guidelines for selecting Enhancer

- **Enhancers for blotting assays**
  There are two enhancers for use in blotting applications, Nitro-Block™ Enhancer and Nitro-Block-II™ Enhancer.

  Table 1 Summary of Enhancer use based for blotting assays

Substrate	Membrane	Enhancer
CSPD™ substrate	Nitrocellulose	Nitro-Block™ or Nitro-Block-II™
	PVDF	Nitro-Block™ or Nitro-Block-II™
	Nylon	–
CDP-Star™ substrate	Nitrocellulose	Nitro-Block-II™
	PVDF	–
	Nylon	–

- **Enhancers for solution assays**
  For solution-based assays such as ELISA, the use of a chemiluminescence enhancer is necessary with CSPD™ and CDP-Star™ substrates. Two enhancers, Emerald-II™ Enhancer and Sapphire-II™ Enhancer, are available for use in these types of assays.

  Table 2 Summary of Enhancer use based for solution assays

Enhancer	Substrate	λ_{max}	Optimal application
Emerald-II™ Enhancer	CDP-Star™ substrate or CSPD™ substrate	542 nm	Emerald-II™ Enhancer-containing substrate formulations may yield improved results on CCD-camera-based imaging systems.
Sapphire-II™ Enhancer		461 nm	Sapphire-II™ Enhancer-containing substrate formulations may perform better on photo-multiplier tube-based detection systems, which are more sensitive to blue light.

Guidelines for diluting substrate and enhancer concentrates

The recommended working concentration of the ready-to-use formulations ensures a linear relationship between signal intensity and enzyme concentration within the nanogram to femtogram range, based on the biochemical and kinetic properties of AP and the substrates.

Note: Use of substrate concentrations lower than that recommended may result in non-linear relationships, making interpretation of results more difficult.

- For **solution-based assays**, use substrates and enhancers as follows:
  - Use CSPD™ substrate or CDP-Star™ substrate at a concentration of 0.4 mM.
  - Use Sapphire-II™ Enhancer or Emerald-II™ Enhancer at a final concentration of 10% (v/v).
  - Dilute CSPD™ substrate (provided as a 25 mM solution) by 62.5-fold to achieve a final concentration of 0.4 mM.
  - Dilute CDP-Star™ substrate (provided as a 12.5 mM solution) by 31-fold to obtain a final concentration of 0.4 mM.
- For **blotting assays**, use substrates and enhancers as follows:
  - Use CSPD™ substrate or CDP-Star™ substrate at a concentration of 0.25 mM.
  - Use Nitro-Block™ Enhancer or Nitro-Block-II™ Enhancer at a final concentration of 5% (v/v).
  - Dilute CSPD™ substrate (provided as a 25 mM solution) by 100-fold to achieve a final concentration of 0.25 mM.
  - Dilute CDP-Star™ substrate (provided as a 12.5 mM solution) by 50-fold to obtain a final concentration of 0.25 mM.

Table 3 Consolidated dilution data summary

Description	CSPD™ substrate (25 mM concentrate)		CDP-Star™ substrate (12.5 mM concentrate)	
	Solution based assay	Blotting based assay	Solution based assay	Blotting based assay
Working concentration	0.4 mM	0.25 mM	0.4 mM	0.25 mM
Dilution Buffer	0.1 M Diethanolamine containing 1 mM $MgCl_2$			
Blocking Buffer	1X PBS with 0.2% casein (recommended working concentration)			
pH	9.5	10	9.5	8.5
Substrate Dilution Factor	62.5	100	31	50
Enhancer	Emerald-II™ or Sapphire-II™	Nitro-Block™ or Nitro-Block-II™	Emerald-II™ or Sapphire-II™	Nitro-Block-II™
Enhancer concentration	10% (v/v)	5% (v/v)	10% (v/v)	5% (v/v)
Example volume to make 10 mL of working solution				
Dilution Buffer volume	8.84 mL	9.4 mL	8.68 mL	9.3 mL
Substrate volume	0.16 mL	0.1 mL	0.32 mL	0.2 mL
Enhancer volume	1 mL	0.5 mL	1 mL	0.5 mL

Before you begin

- Use deionized water to prepare all solutions.
- Keep 10X PBS / TBS sterile at room temperature.
- Prepare buffers fresh daily to prevent bacterial contamination.
- Store Blocking Buffer at 4°C if 0.02% NaN_3 is added.
- Ready-to-Use substrate formulations are provided in an alkaline buffer solution, and it is recommended that substrate concentrates be diluted in an alkaline buffer.

Perform assay

Procedure for solution-based assays (immunoassay or ELISA)

We focus on the direct sandwich immunoassay format, known for its high sensitivity in detecting soluble antigens, among the various immunoassay formats available including both competitive and sandwich immunoassay formats. This format is recommended for antigens for which two distinct antibodies are available.

1. Coat plate with capture antibody, then wash coated plate 3 times with Wash Buffer.
2. Incubate wells with Blocking Buffer at room temperature, then wash blocked plate 3 times with Wash Buffer.
3. Dilute antigen samples in Blocking Buffer, then add 100 µL of antigen samples into the wells.
4. Incubate for 1 hour at room temperature with shaking. Wash wells 3 times with Wash Buffer.
5. Dilute detector antibody-AP conjugate in Blocking Buffer, then add 100 µL of detector antibody-AP conjugate into the wells.
6. Incubate for at least 1 hour at room temperature with shaking. The optimal conjugate dilution must be empirically determined.
7. Wash wells 4 times with Wash Buffer then twice with 1X Assay Buffer.
8. Add 100 µL of Substrate/Enhancer Solution into the wells.
9. Incubate for 5–10 minutes at room temperature, then measure at 5-minute intervals until peak light emission occurs (typically 20–30 minutes after substrate addition at room temperature (18–25°C).

Procedure for blotting-based assays

The protocol is for western blotting on a nitrocellulose membrane.

IMPORTANT!

- The indicated volumes are for a single blot (100 cm^2) and should be adjusted for larger or smaller blots.
- All steps are performed at room temperature (18–25°C) with agitation.

1. After transferring, briefly rinse the blot with PBS or TBS, and incubate in a minimum of 10 mL of Blocking Buffer for 30–60 minutes.

   Note: PBS or TBS buffers can be used with either nitrocellulose or PVDF membranes. With nylon, background is slightly higher with TBS than with PBS.

2. Dilute primary antibody in Blocking Buffer (5–10 mL), then add the antibody into the blot.

3. Incubate the blot for 30–60 minutes, then wash at least 2 times for 5 minutes each.

   - For nitrocellulose and nylon, use Wash Buffer to wash.
   - For PVDF, use Blocking Buffer to wash.

   Note: Use at least 20 mL for all washes.

4. Dilute secondary antibody-AP conjugate 1:5,000 in Blocking Buffer (5 mL), then add into the blot. Incubate the blot for 30–60 minutes.

5. Wash 3 times for 5 minutes each as in step 3, then rinse 2 times for 2 minutes each with 1X Assay Buffer.

6. Drain blots by touching a corner on a paper towel, then place on plastic wrap on a flat surface (do not let blots dry).

7. Pipette a thin layer of substrate solution (3 mL) onto the blot, then incubate for 5 minutes.

   - For nitrocellulose and PVDF membranes, use CSPD™ substrate with Nitro-Block™ Enhancer.
   - For nitrocellulose membranes, use CDP-Star™ substrate with Nitro-Block-II™ Enhancer (150 µL enhancer with 3 mL of substrate solution for 100 cm^2 of membrane).

   IMPORTANT! Avoid using enhancer on PVDF with CDP-Star™ substrate or on nylon with either substrate.

8. Drain excess substrate solution and place blot in development folder (after removing anti-static sheet) or wrap in plastic. Smooth out bubbles or wrinkles.

9. Image the blots by placing them in contact with standard X-ray film.

   Note: We recommend initiating exposures for 5–30 minutes. Exposure times with PVDF or nylon membranes are usually shorter than with nitrocellulose.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000298 A

Revision	Date	Description
A	11 November 2024	New document for CSPD™ and CDP-Star™ Substrates.