

WESTERN LIGHTNING™

CDP-Star® Chemiluminescence Reagent (Ready-to-Use)
125 mL for 2500 cm²-12,500 cm², 50 mL for 1000 cm²-5,000 cm²

Catalog Number:	NEL602, NEL602A
Shipping Conditions:	Ambient Temperature
Storage Conditions:	4°C

Product Description

Western Lightning™ CDP-Star Chemiluminescence Reagent (Ready-to-Use) contains the chemiluminescence substrate CDP-Star (MW=495.2) for the detection of alkaline phosphatase. **The reagent, prepared in a proprietary buffer, is ready to use and does not require any further dilution.**

Introduction

CDP-Star is a non-radioactive light emitting reagent used to detect the enzyme alkaline phosphatase immobilized on a membrane. Maximum light emission occurs two hours after substrate incubation with continuous light output for a minimum of 24 hours (See Figure 1).

When used in Western Blotting applications, picogram quantities of protein can be detected. Film or *KODAK* Image Station exposure to achieve this level of sensitivity can be 1 second to 10 minutes.

Light Emission Kinetics of CDP-Star® on Nylon Membrane

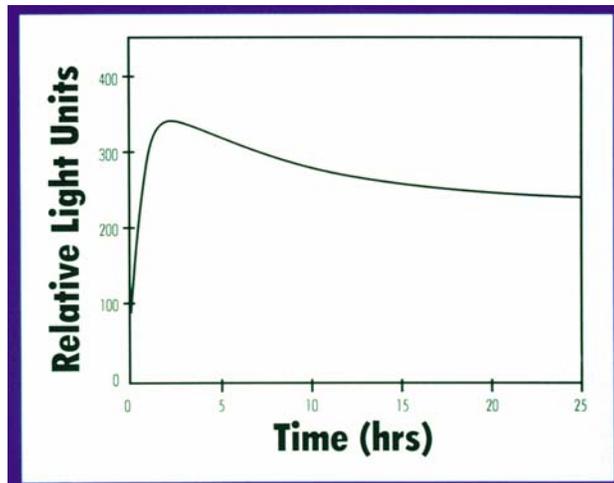


Figure 1

Intended Use

Intended use of this product is the detection of alkaline phosphatase.

Performance Considerations

1. This reagent has been formulated and quality controlled specifically to detect alkaline phosphatase antibody or streptavidin conjugates immobilized onto membranes.
2. Western Lightning™ CDP-*Star* Chemiluminescence Reagent (Ready-to-Use) is applicable to PVDF membranes such as PolyScreen® PVDF Transfer Membrane and also nylon membranes such as GeneScreen™ and GeneScreen *Plus*®.
3. KODAK X-OMAT Blue Film or the *KODAK* Image Station, ideal for chemiluminescence CDP-*Star* detection, are recommended for best results.

Protocol for Use

The following is a *protocol* for using CDP-*Star* in a Western Blot experiment. The PolyScreen PVDF Transfer Membrane product insert contains detailed protocols for membrane wetting and Western Blotting.

1. If PolyScreen has dried after completion of Western Blotting procedure it must be re-wet with 95% ethanol for at least one minute. Rinse the membrane in distilled water to wash off the alcohol for 2-3 minutes.
2. Block the membrane in Blocking Buffer [PBST (0.05M phosphate, 0.154M NaCl, 0.05% TWEEN 20)] for 1 hour at room temperature. To prepare blocking buffer add blocking reagent slowly to the PBST while stirring. Continue stirring and heat the solution slowly to 60°C until all of the blocking reagent is dissolved. The resulting solution will be opaque. Cool to room temperature before use. Alternatively, the membrane may be blocked in 5% non-fat dry milk in PBST.
3. Dilute primary antibody in Blocking Buffer and incubate for at least 1 hour at room temperature.
4. Wash membrane 2 X 5 minutes in Blocking Buffer at room temperature.
5. Dilute secondary antibody in Blocking Buffer and incubate for at least 1 hour at room temperature.
6. Wash membrane 3 X 5 minutes in Blocking Buffer at room temperature.
7. Mark the membrane to distinguish the side with the immobilized alkaline phosphatase and wash for 5 minutes in 0.1 M Tris (pH 9.5).
8. Place the membrane in a clean dish or hybridization bag. Carefully add CDP-*Star* to membranes (0.01 mL/cm²-0.05 mL/ cm²). Incubate for 5 min at room temperature with agitation. The blot should be covered with solution during this incubation.
9. Remove the blot from the substrate and gently blot off any excess reagent onto any filter paper or paper towels. **Do not allow the membranes to dry.**
10. Place the membranes, protein side up, between plastic sheet protectors (Boise Cascade Catalog Number L2-A8112) or in plastic wrap and expose initially to KODAK X-OMAT Blue film or the *KODAK* Image Station for 5-10 minutes. Re-exposures can be done until optimal signal to noise ratios are achieved as chemiluminescence signal from CDP-*Star* continues for at least 24 hours.

Stability

The reagent is stable for at least one year from the date of manufacture when stored properly at 4°C.

Storage

Western Lightning™ CDP-*Star* Chemiluminescence Reagent (Ready-to-Use) is shipped ambient and should be placed at 4°C upon arrival for maximum stability.

Safety Considerations

Caution: For Laboratory Use. A Research Chemical for Research Purposes Only.

Warning: Harmful by Contact, Ingestion, Inhalation. Irritating to Eyes, Skin, & Respiratory Tract; Slightly Toxic.

Target Organs: Eyes, Upper Respiratory Passages, Skin

Trademarks

CDP-*Star* is a registered trademark of Applied Biosystems, Inc. CDP-*Star* is protected under one or more of U.S. Patents 5,326,882; 4,931,569; 5,582,980; 5,851,771; 5,538,847; 5,145,772; 4,978,614.