molecular probes®
by life technologies™

DynaLight® Substrate with RapidGlow® Enhancer and DynaLight® Trigger Solution

Table 1 Contents and storage

Material	Cat. no.	Amount	Storage*	Stability†
DynaLight® Substrate with RapidGlow® Enhancer	4475406	100 mL	2–8°C	• When stored at 2–8°C, the product is stable for 1 year. • When stored at room temperature (15–25°C), the product is stable for at least 6 months.
	4475410	1 L		
DynaLight® Trigger Solution	4475403	100 mL		
	4475409	1 L		

* Store DynaLight® Substrate with RapidGlow® Enhancer and DynaLight® Trigger Solution at 2–8°C for long-term storage.

† DynaLight® Substrate with RapidGlow® Enhancer may turn cloudy in appearance at elevated temperatures, but this does not affect product performance. Material will return to original appearance when stored at 2–8°C.

Introduction

DynaLight® Substrate with RapidGlow® Enhancer is a chemiluminescent formulation which can emit light when catalyzed by purified alkaline phosphatase enzyme or by alkaline phosphatase enzyme conjugated to macromolecules such as antibodies, nucleic acids, or antigens.

Alkaline phosphatase enzyme or moieties/labels enable substrate decomposition by cleaving phosphate group and signal amplification due to the high turnover rate. Further decomposition of the intermediate substrate results in an ester moiety in high excited state; as this ester moiety goes to ground state it emits light, which is detectable by a luminometer instrument. Light emission resulting from the chemical decomposition of substrate exhibits an initial delay to reach maximum light emission, which lasts from 2–10 minutes depending in experimental conditions such as temperature, enzyme activity, and enzyme label environment, followed by a sustained maximum light emission that can last for hours. DynaLight® Substrate with RapidGlow® Enhancer reaches 80–90% of maximum light emission within the first few seconds after substrate addition to the enzyme or the enzyme label well. For optimal performance, DynaLight® substrate requires an alkaline hydrophobic environment and the RapidGlow® enhancer, which protects the emitter from quenching the effects of water environment. The DynaLight® Substrate is provided as a ready-to-use solution of substrate with RapidGlow® enhancer in alkaline hydrophobic environment for assays. Relative light units (RLUs) produced by the chemiluminescent reaction can be measured by a luminometer.

Immunoassay detection using chemiluminescent 1,2-dioxetane substrates requires an alkaline phosphatase (AP) enzyme conjugate to trigger the decomposition of the 1,2-dioxetane substrate, followed by light emission. In a sandwich immunoassay format, AP is conjugated to either a primary or secondary antibody, while in a competitive immunoassay format it is conjugated to the antigen of the antibody. While the alkaline phosphatase enzyme conjugate triggers the initiation of 1,2-dioxetane substrate decomposition, a solution with a high pH such as the DynaLight® Trigger Solution or NaOH trigger (e.g. Trigger 1 used in chemiluminescent label substrates such as isoluminol or acridinium ester conjugates) can accelerate decomposition even further, resulting in fast results and an enhanced signal.

Dioxetane substrates can be divided in 2 groups based on their signal kinetics, "Glow" and "Flash and Glow" substrates. "Glow" substrates, such as CDP-Star® and CSPD® substrates, provide a sustained maximum signal over time only after 15–60 minutes depending on the temperature, while "Flash and Glow" substrates, such as the DynaLight® substrate, provide a fast and sustained maximum signal as early as 2–10 minutes depending on the temperature. CDP-Star® Substrate with Sapphire-II™ (or Emerald-II™) Enhancer (i.e., "Glow" substrate formulation) and DynaLight® Substrate with RapidGlow® Enhancer (i.e., "Flash and Glow" substrate formulation) can each be used in several different reading formats due to their high signal as early as the first minute (the latter providing faster maximum signal than the former) and their compatibility with high pH-ed solutions. In instruments that enable inject-and-read parameters, 1,2-dioxetanes can be injected as 1-reagent (substrate alone) or as 2-reagents (substrate reagent plus a triggering solution such as the DynaLight® Trigger Solution) to achieve readings as early as seconds to minutes after substrate injection.

Guidelines for Use

DynaLight® Substrate with RapidGlow® Enhancer and DynaLight® Trigger Solution offer flexibility of detection with any chemiluminescent instrumentation, throughput format and solid-phase assay. For example, in a bead-phase assay, when DynaLight® Substrate with RapidGlow® Enhancer is assayed at room temperature (~25°C), maximum signal is reached within ~5–10 minutes and this signal is sustained for hours (see Figure 1, page 3). When assayed at 37°C, the signal intensity reaches plateau earlier, at around 2 minutes, and this signal is also sustained for hours. At either temperature, the signal-to-noise ratio remains constant over time, enabling read times as early as 1 minute for instrumentation with inject-and-read parameters. Alternatively, waiting for the signal intensity to reach a plateau at around 10 minutes at room temperature allows the reading of the entire plate at 1 second/well for instrumentation that enables whole 96- or 384-well plate reading. As a comparison, it takes CDP-Star® Substrate with Sapphire-II™ Enhancer up to 1 hour to reach maximum signal at room temperature, and 15 minutes at 37°C.

Choosing the Correct Detection Protocol

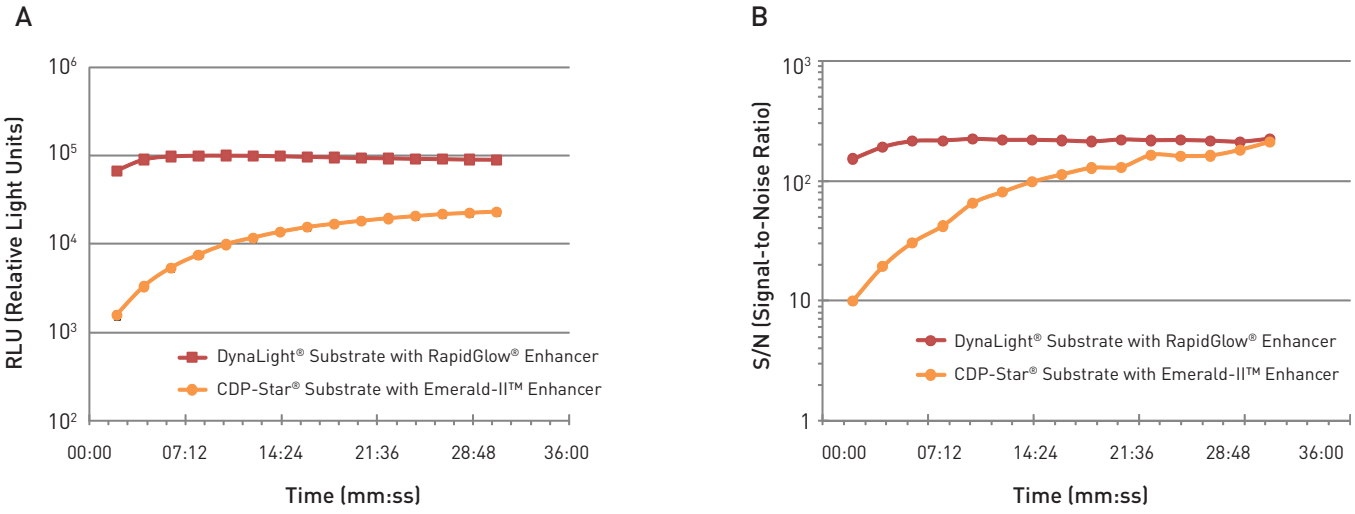
When using the product within a given instrument, throughput format, or detection of alkaline phosphatase moiety in a solution-, membrane- or solid-phase assay (i.e., microplate or bead-based immunoassays, membrane-based protein detection, microplate or membrane based nucleic acid detection etc.), choose the correct detection protocol based on the light emission kinetics (see **Detection Protocols**, page 5).

- When DynaLight® Substrate with RapidGlow® Enhancer is used as a single reagent in a 96-throughput assay format (or more), follow the **96-well Microplate Reading Format** protocol.
- When DynaLight® Substrate with RapidGlow® Enhancer is used as a single reagent in a single assay throughput format, follow the **1-Injection Reading Format** protocol.
- When DynaLight® Substrate with RapidGlow® Enhancer is used together with DynaLight® Trigger Solution, follow the **2-Injections Reading Format** protocol.

"Flash and Glow" Light Emission Kinetics

"Flash and Glow" substrates, such as the DynaLight® Substrate with RapidGlow® Enhancer, provide a fast and sustained maximum signal as early as 2–10 minutes depending on the temperature. Figure 1, below, compares the signal intensity kinetics (panel A) and the stability over time of the signal-to-noise ratios (panel B) of "Glow" and "Flask and Glow" substrate formulations (i.e., CDP-Star® Substrate with Emerald-II™ Enhancer and DynaLight® Substrate with RapidGlow® Enhancer, respectively).

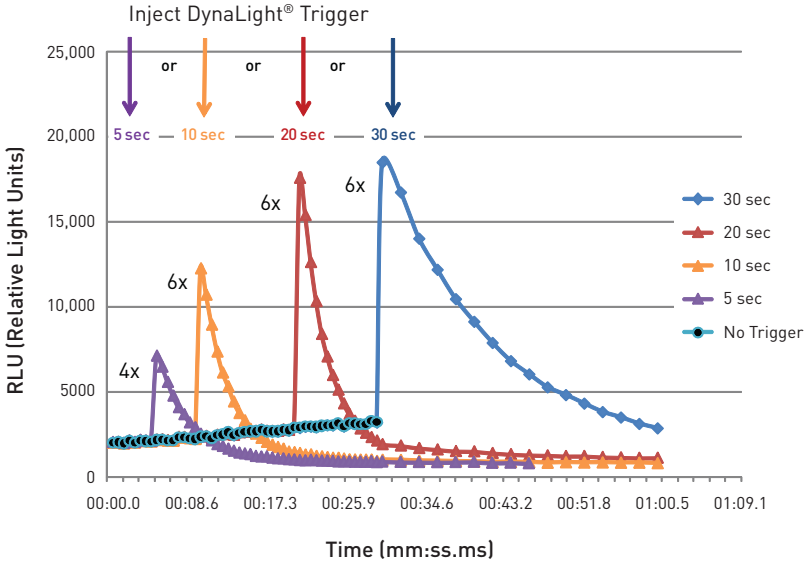
Figure 1 1-Injection Signal Kinetics at 25°C, comparing signal intensity kinetics (panel A) and signal-to-noise ratios (panel B) of "Glow" and "Flash and Glow" substrate formulations. 0.04 ng of biotin-alkaline phosphatase was detected using 20 µg of M-280 SA Dynabeads® magnetic beads in a white microplate. Signal kinetics at 25°C are shown for both "Glow" (i.e., CDP-Star® Substrate with Emerald-II™ Enhancer) and "Flash and Glow" (i.e., DynaLight® Substrate with RapidGlow® Enhancer) substrates, respectively reaching maximum light emission at ~30 minutes and ~5–10 minutes.



"Flash" Light Emission Kinetics

Time to results can be shortened to less than 30 seconds without compromising signal intensity when DynaLight® Substrate with RapidGlow® is used together with DynaLight® Trigger Solution. In order to use both reagents, the instrument should have two injectors on board and the capability to inject and read each assay individually.

Figure 2 2-Injections Signal Kinetics. After the last step of cardiac Troponin-I bead immunoassay, washed beads were incubated for time intervals of 5, 10, 20, and 30 seconds with DynaLight® Substrate and RapidGlow® Enhancer, after which DynaLight® Trigger Solution was injected and the signal read from 5, 10, 20, and 30 seconds to 1 minute and 40 seconds timeframe. Signal from 0.192 ng/mL of cardiac Troponin-I is shown to illustrate flexibility of usage at any given time point when DynaLight® Substrate and RapidGlow® Enhancer is used with DynaLight® Trigger Solution.



Recommended Buffers and Solutions for Use in Assays

AMP, DEA (diethanolamine), or carbonate buffers, pH 9.5 (with $MgCl_2$ traces needed for the alkaline phosphatase) are optimal buffers for use with 1,2-dioxetane alkaline phosphatase substrates. For solution based assays, such purified alkaline phosphatase enzyme assays, the same recommended buffers should be used for diluting the enzyme to the desired level. Also, for solution based assays the enzyme dilution volume to substrate volume ratio is recommended to be approximately 1:10; for example, 10 µL of enzyme dilution with 100 µL of substrate/enhancer formulation.

Detection Protocols

96-well Microplate Reading Format

96-well microplate reading format is recommended for microplate based chemiluminescent instruments and for assays performed using the entire 96-well microplate.

1. Complete the final wash step of your immunoassay procedure.
2. Dispense 100 µL DynaLight® Substrate with RapidGlow® Enhancer to 96-well microplate (the volume can be increased or decreased as appropriate), following the same reading patterns as the reading protocol set up on the instrument.

   For example, if the instrument reads in a column-wise direction, then dispense 100 µL of DynaLight® Substrate with RapidGlow® Enhancer to the microplate in a column-wise fashion using a 12-channel pipettor. If the instrument reads in a row-wise direction, then dispense 100 µL of DynaLight® Substrate with RapidGlow® Enhancer to the microplate in a row-wise fashion using an 8-channel pipettor.
3. Incubate the plate for 5–10 minutes, depending on the incubation temperature (~5 minutes at 37°C or 10 minutes at room temperature).
4. Read the entire plate for 1 second per well.

1-Injection Reading Format

1-injection protocol is recommended for inject-and-read based chemiluminescent platforms utilizing an injector and a single well, cuvette, or tube assay:

1. Complete the final wash step of your immunoassay procedure.
2. Inject 100 µL of DynaLight® Substrate with RapidGlow® Enhancer to the well, cuvette, or tube (the volume can be increased or decreased as appropriate).
3. Incubate for 1 minute (the incubation time can be decreased or increased as appropriate).
4. Read for 1 second per well, cuvette, or tube.

2-Injections Reading Format

2-injections reading format includes DynaLight® Substrate with RapidGlow® Enhancer reagent and DynaLight® Trigger Solution. This protocol is recommended for instruments with trigger solutions already in place, which can be replaced with DynaLight® Substrate with RapidGlow® Enhancer reagent and DynaLight® Trigger Solution.

1. Complete the final wash step of your immunoassay procedure.
2. Inject 50 µL of DynaLight® Substrate with RapidGlow® Enhancer to the tube.
3. Incubate for 1 minute (this can be increased or decreased as appropriate).
4. Add 50 µL of DynaLight® Trigger Solution.
5. Read for 0.6 second per tube (0.6 seconds is typical peak signal after DynaLight® Trigger addition whereas the typical half life of signal is ~10 seconds after trigger addition; this can be increased or decreased based on specific assay signal kinetics).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
4475406	DynaLight® Substrate with RapidGlow® Enhancer	100 mL
4475410	DynaLight® Substrate with RapidGlow® Enhancer	1 L
4475403	DynaLight® Trigger Solution	100 mL
4475409	DynaLight® Trigger Solution	1 L

Purchaser Notification

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