

Novel DynaLight™ Substrate with RapidGlow™ Enhancer: 1,2-Dioxetane Formulation for Application in Clinical Research Platforms.



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OVERVIEW

- Life Technologies' DynaLight™ Substrate with RapidGlow™ Enhancer chemiluminescent formulation:
- ❖ Application:
 - ✓ Clinical research immunoassays using 'Flash' or 'Glow' detection
 - ❖ Results:
 - ✓ Flexible – 'Flash' or 'Glow' signal:
 - ✓ Flash signal generation in seconds with DynaLight™ Trigger Solution
 - ✓ Glow signal level achieved in minutes and remains stable for hours, ideal for microplate ELISAs
 - ✓ Sensitive - signal amplification with alkaline phosphatase conjugates, combined with low background signal of the chemiluminescent substrate produces the highest sensitivity and widest dynamic range
 - ✓ Optimized with Dynabeads® products for optimal combined performance
 - ✓ Robust - Substrate formulation is stable for > 7 months and counting at 4°C

INTRODUCTION

We demonstrate the enhanced performance of a novel 1,2-dioxetane alkaline phosphatase substrate, DynaLight™ Substrate, designed for combination of the greatest characteristics of both substrate and enzyme label detection systems: **fast results with amplified signal for ultra-sensitive detection in clinical research platforms.**

DynaLight™ Substrate with RapidGlow™ Enhancer formulation was selected for commercialization based upon performance attributes (i.e. fast kinetics, high signal, low background, high sensitivity and thermal stability) obtained from enzyme assays and further validated in microplate- and bead-based immunoassay model assays.

In addition, DynaLight™ Substrate offers the flexibility of coupling the enzymatic catalysis with a subsequent DynaLight Trigger Solution which raises the pH and velocity of reaction, to provide **signal 'on demand'** - very useful for automated platforms when faster test protocols are employed or when there is a need to switch to new reagents with trigger positions already in place.

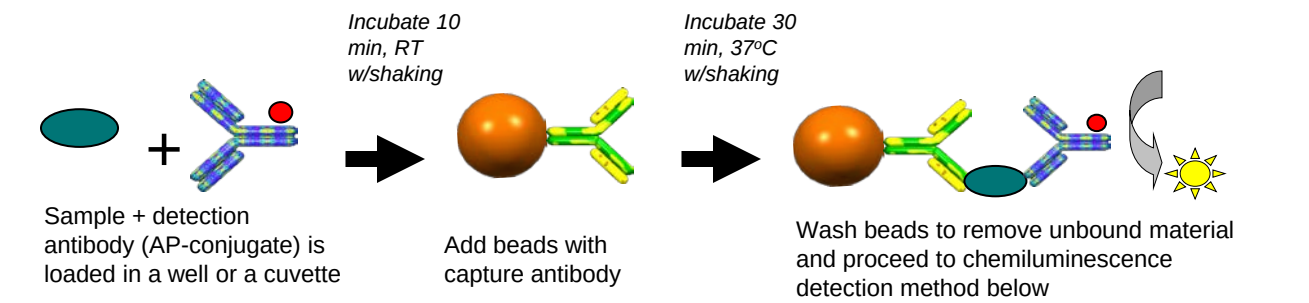
MATERIALS AND METHODS

Detection reagents: Life Technologies': DynaLight™ Substrate with RapidGlow™ Enhancer, DynaLight™ Trigger, CDP-Star® Enhancer with Emerald-II™ Enhancer, CDP-Star® Substrate with Sapphire-II™ Enhancer. Vendor's: Fast Alkaline Phosphatase (AP) Substrates.

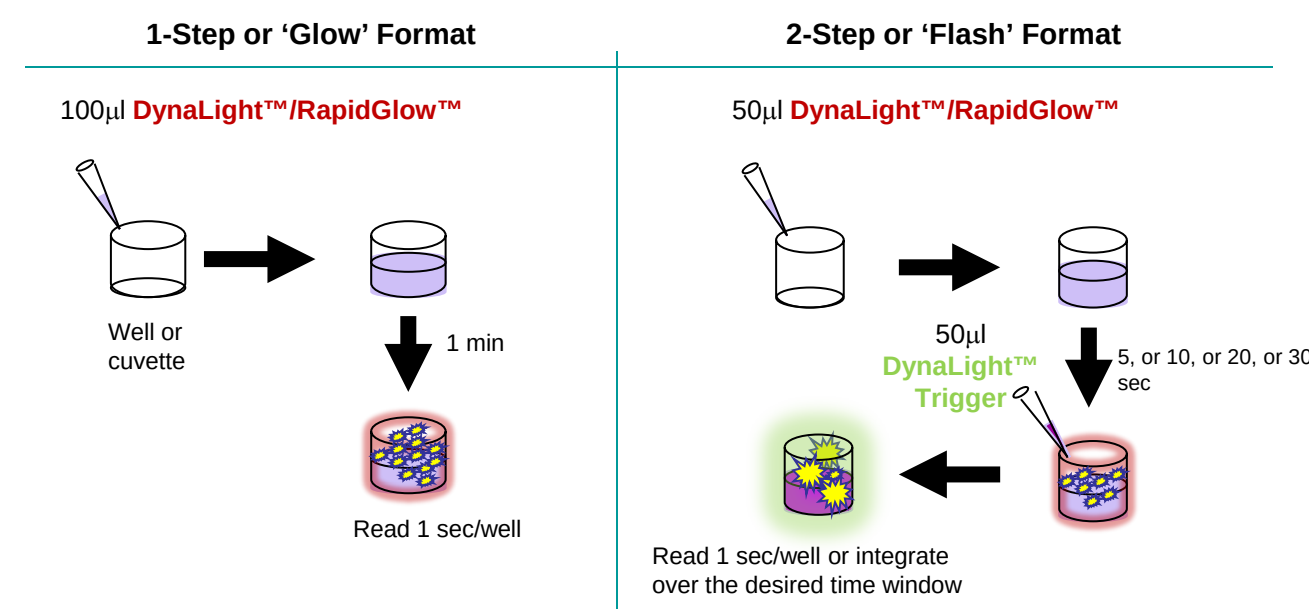
Troponin-I immunoreagents: M-280 Tosylactivated Dynabeads® magnetic beads (Life Tech, 14204) are coupled to α-Troponin-I antibody (SDIX, a.a.r. 24-40, clone TPC-110, P/N B9085MA06-MA), alkaline phosphatase enzyme (LYNX Rapid Alkaline Phosphatase Antibody Conjugation Kit (LNK021AP) is coupled to α-Troponin-I antibody (HyTest Ltd., 4T21 Mab pair 560-19C7), Troponin-I (HyTest Ltd., 8T53).

Estradiol competitive microplate immunoassay: Commercially available 17β-estradiol luminescence immunoassay kit (IBL Int., RE62041).

Dynabeads® magnetic beads Troponin I sandwich ELISA method schematic:



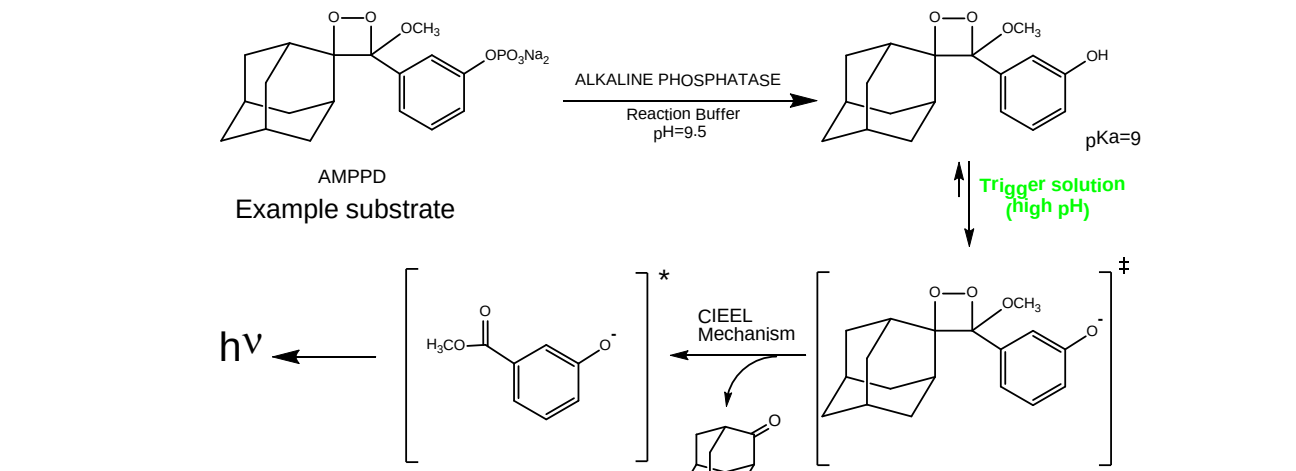
Chemiluminescence detection method schematics:



RESULTS

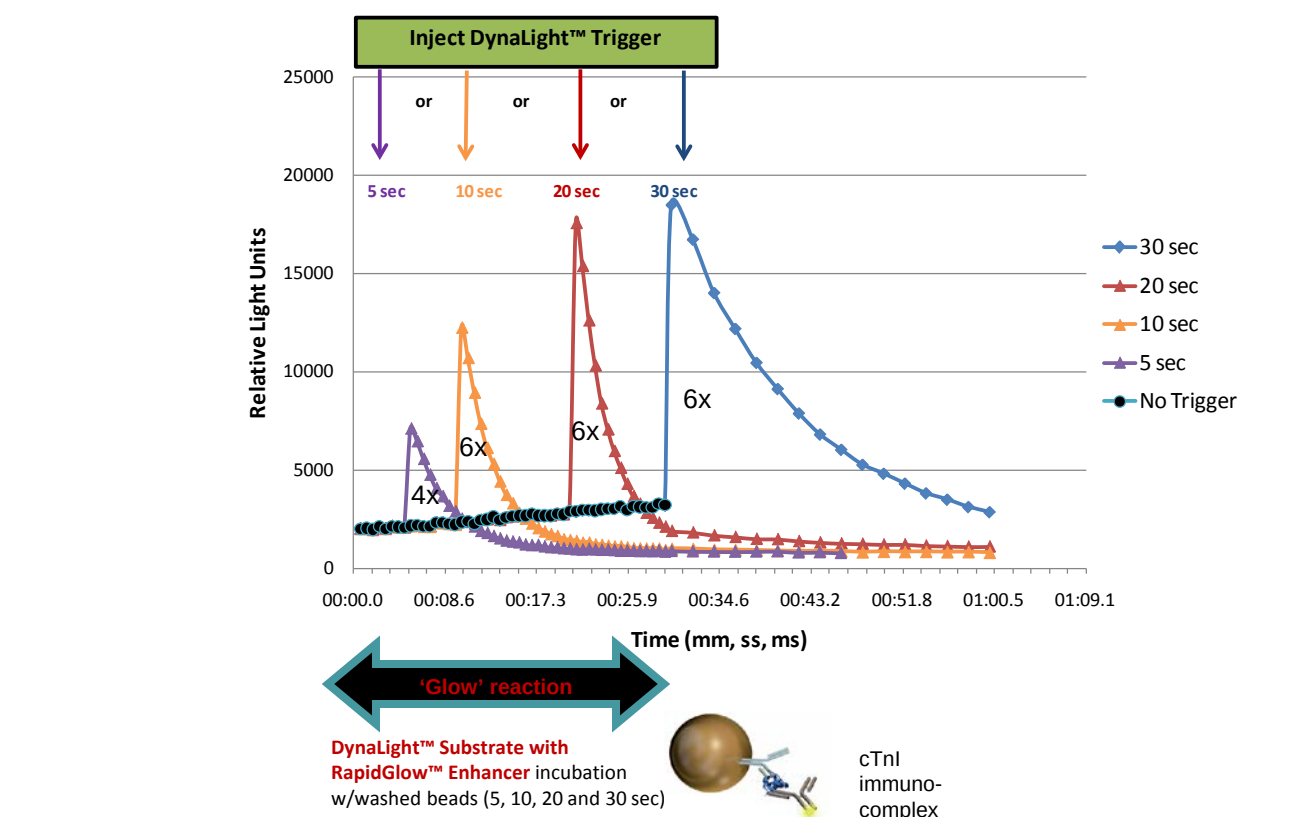
Detection Flexibility: Flash and Glow Light Emission

Figure 1. Mechanism Behind 'Flash' and 'Glow' Kinetics of 1,2-Dioxetanes



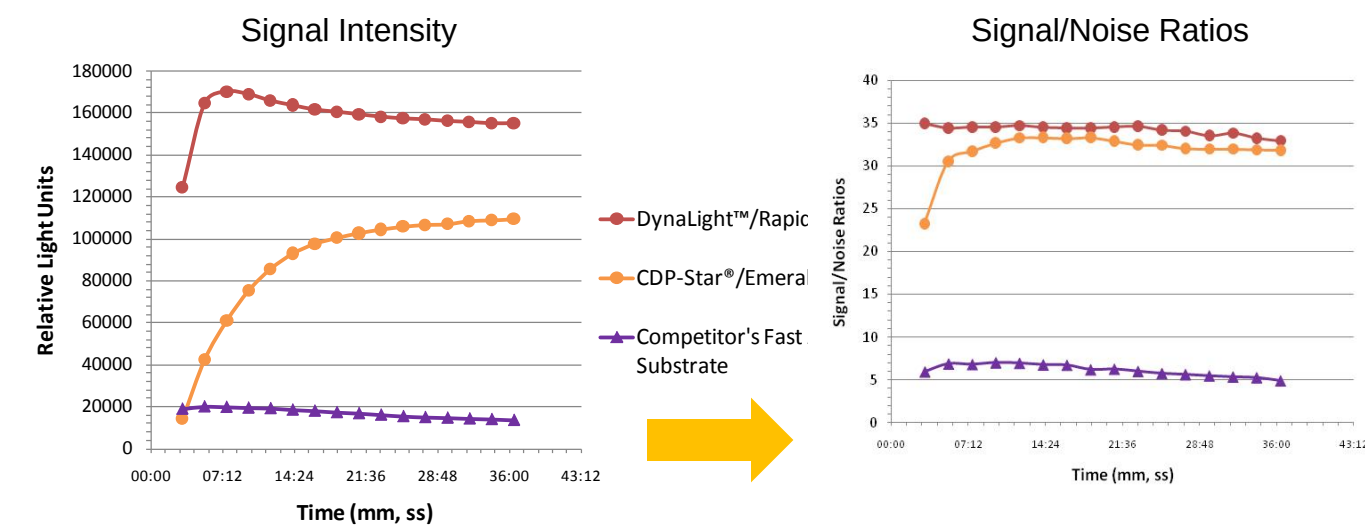
Alkaline phosphatase (AP) enables substrate decomposition and signal amplification due to the high turnover rate. Further decomposition of the metastable phenolate is speeded up by high pH DynaLight™ Trigger solution to produce an ester moiety in high excited state, which emits light when it goes to ground state, detectable by a luminometer instrument.

Figure 2. Flash Kinetics – Signal “On Demand” with DynaLight™ Trigger in Bead Immunoassay, at 30°C



Signal kinetics before and after DynaLight™ Trigger addition. After the last step of immunoassay, washed beads were incubated for time intervals of 5, 10, 20 and 30 sec with DynaLight™ Substrate and RapidGlow™ Enhancer and then DynaLight™ Trigger Solution was injected and signal read for 1 min and 40 seconds timeframe. 30 sec incubation with enzyme has been used for all trigger protocols shown in this poster, unless otherwise indicated.

Figure 3. Glow Kinetics –Signal after DynaLight™/RapidGlow™ Formulation Addition in Bead Immunoassay, at 30°C

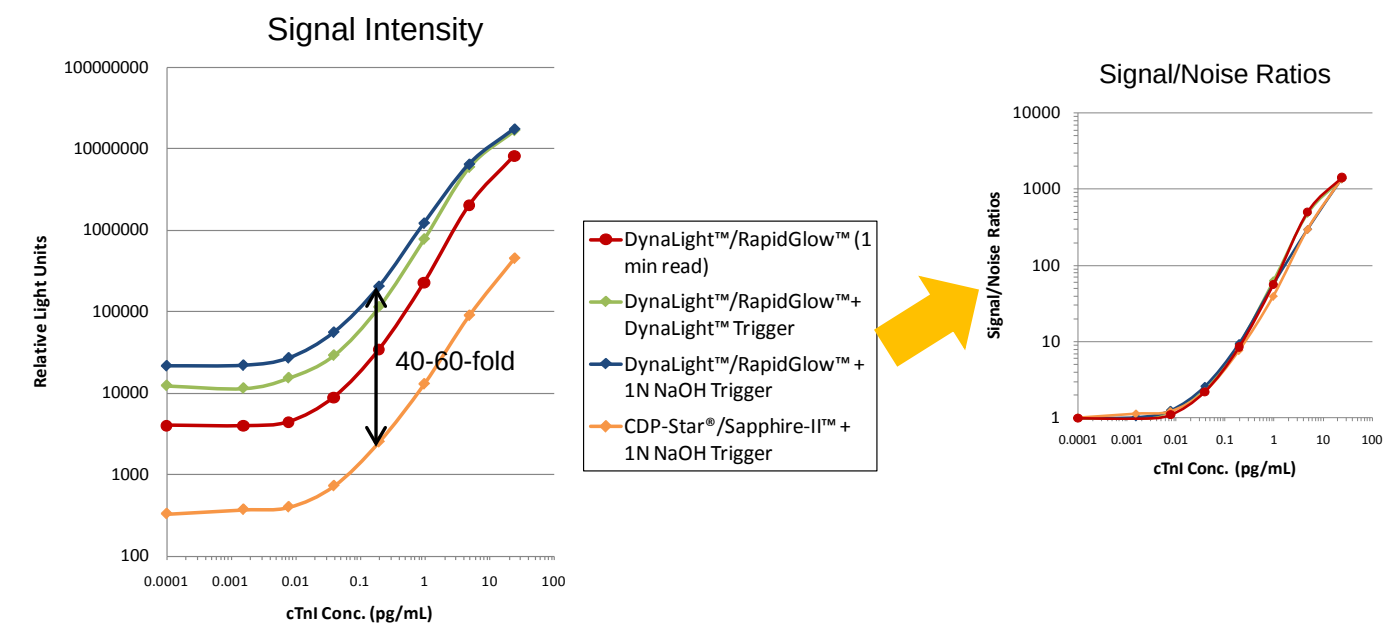


Signal kinetics of DynaLight™ Substrate with RapidGlow™ Enhancer shows maximum light emission, as early as 7 min, permitting reading large number of samples for a brief period.

Signal to noise ratios of DynaLight™ Substrate with RapidGlow™ Enhancer show stable signal/noise ratios over time. The new Life Technologies' substrate outperforms leading competitor's substrate in signal to noise ratios.

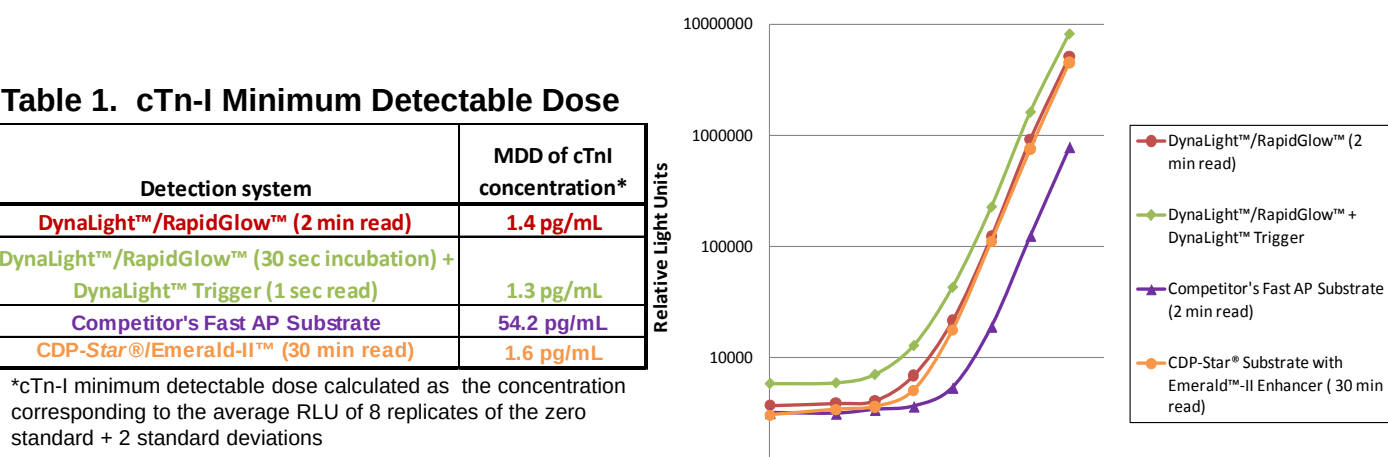
Assay Dynamic Range and MDD (Minimum Detectable Dose)

Figure 4. Multiple Approaches for Triggering – Assay Dynamic Range with DynaLight™ Trigger vs. NaOH Trigger in Bead Immunoassay, at 30°C



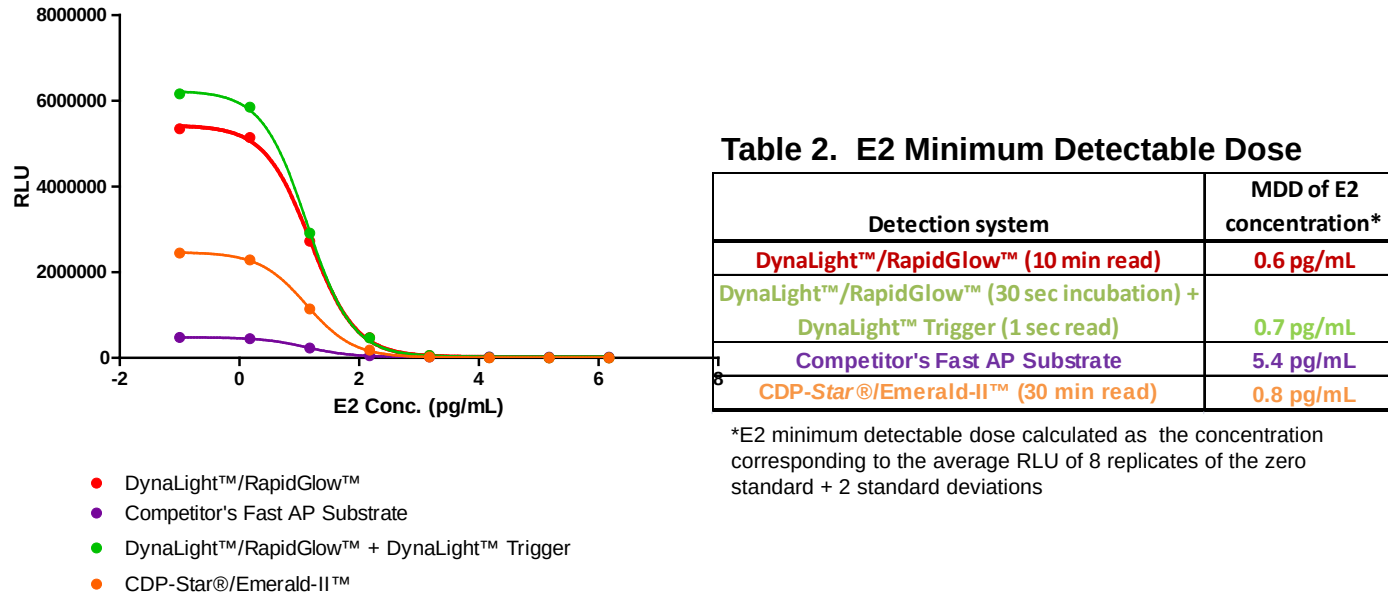
While different signal intensity are seen with different substrates (~40-60—fold range), no significant signal intensity differences are seen between different trigger solutions, demonstrating compatibility of DynaLight™ with multiple Trigger solutions and formats. Independent of substrate, trigger or enhancer, the signal/noise ratios are almost the same.

Figure 5. Cardiac Troponin-I Bead Immunoassay Sensitivity Comparison to Other Detection Chemistries, at 30°C



Wide dynamic range of about 4 orders of magnitude and higher signal intensity is exhibited by DynaLight™/RapidGlow™ and/or DynaLight™ Trigger, standing out on MDD (see table 1) when compared to competitor's AP substrate while matching performance of CDP-Star®/Emerald-II™.

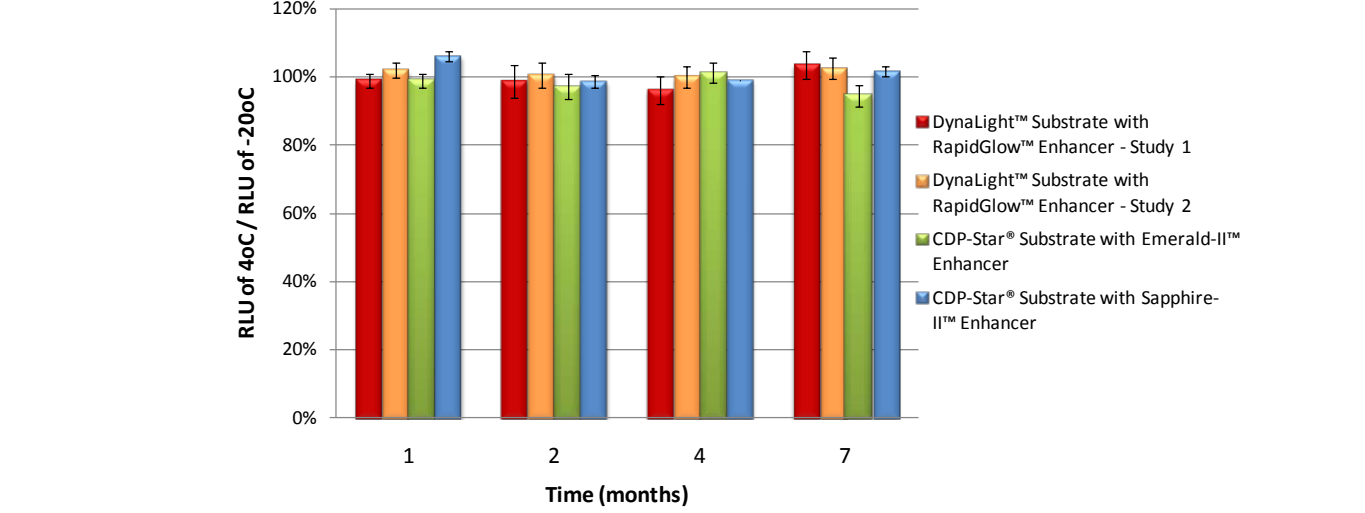
Figure 6. Robust Results with Other Immunoassay Formats – Competitive 17β-Estradiol Luminescence Immunoassay Format, at 25°C



Wide dynamic range of about 4 orders of magnitude and higher signal intensity is exhibited by DynaLight™/RapidGlow™ and/or DynaLight™ Trigger standing out on MDD (see table 2) when compared to competitor's AP substrate while matching performance of CDP-Star®/Emerald-II™ in a microplate immunoassay format.

Robust Results: Substrate Shelf-Life Stability

Figure 7. Reagents Shelf-Life Stability: 7 Month On-Going Real-Time Study, at 4°C



On-going stress (data not shown) and real-life stability studies for DynaLight™ Substrate with RapidGlow™ Enhancer formulation show shelf-life stability trends of greater than 1 year at 4°C. Signal activity of each ready to use (RTU) formulation stored at 4°C was compared to signal activity of RTU formulations stored at -20°C periodically in a functional assay or immunoassay and % RLU of 4°C sample was compared to RLU of -20°C sample.

CONCLUSIONS

❖ New DynaLight™ Substrate with RapidGlow™ Enhancer provides characteristics of both direct label and enzyme linked chemiluminescent detection systems.

❖ This DynaLight™ Substrate formulation offers fast signal kinetics and slower signal decay than traditional "flash" detection technologies

❖ Enables superior sensitivity, glow signal kinetics and substrate reagent stability that is typical of 1,2-dioxetane substrates.

❖ Assays can be read within minutes (or seconds using DynaLight™ Trigger solution) after substrate addition providing new options for detection platforms requiring early read times and sensitive detection.

❖ The DynaLight Trigger Solution provides fast maximum signal with a slow half-life to ground state allowing ample time to collect data reliably, in addition to being an environmentally friendly reagent.

❖ Glow signal improves assay precision of ELISAs performed in microtiter plates.

❖ DynaLight™ Substrate with RapidGlow™ Enhancer (with or without DynaLight™ Trigger) can detect:

- ❖ attomole amounts of purified alkaline phosphatase in a functional enzyme assay (data not shown).
- ❖ < 1 pg/mL of recombinant human IL-6 in a microplate immunoassay model (data not shown).
- ❖ < 2 pg/mL of cardiac Troponin I in a bead-based immunoassay model employing Dynabeads® magnetic beads and
- ❖ < 1 pg/mL of 17β-estradiol using a commercialized microplate immunoassay kit.

❖ Stability studies for DynaLight™ Substrate with RapidGlow™ Enhancer formulation and DynaLight™ Trigger Solution show shelf-life stability trending towards >1 year at 4°C.

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TRADEMARKS/LICENSING

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