2007 Chemiluminescent Product Guide

Applied Biosystems

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Chemiluminescent Substrates and Chemiluminescent Enhancers

Introduction

Chemiluminescence

Chemiluminescence is the conversion of chemical energy to light energy. Several different chemical reactions, including some enzyme-catalyzed reactions, result in the production of visible light. Chemiluminescence reactions occur naturally (bioluminescence) in a wide variety of organisms, including beetles, jellyfish, bacteria, and many marine organisms. In addition, there are several classes of synthetic chemical structures that upon chemical or enzymatic cleavage produce light emission. Chemiluminescent reactions are employed in a wide variety of applications, including but not limited to biological assays, clinical diagnostic assays, biosensors, hygiene monitoring, and commercial low-level lighting.

Principles of Enzyme-activated Chemiluminescence

1,2-Dioxetane substrates emit visible light upon enzyme-catalyzed decomposition. Chemiluminescent detection of biomolecules with 1,2-dioxetane enzyme substrates is extremely sensitive as a result of low background luminescence coupled with high-intensity light output (due to enzyme cleavage and turnover of a large number of substrate molecules). The energy for light emission is generated internally upon dioxetane decomposition. In comparison, fluorescence requires an external light source for excitation energy, which must be filtered to discriminate the fluorescent signal emission. This limits the sensitivity and introduces complexity into the instrumentation and data analysis.

Glow Kinetics of 1,2-Dioxetanes

The decomposition of CDP-*Star*® substrate is shown in Figure 1. Upon dephosphorylation of the substrate by alkaline phosphatase, a metastable phenolate anion intermediate is formed that decomposes and emits light with a maximum intensity at a wavelength of 475 nm. A delay in reaching maximum light intensity results, the length of which depends upon anion structure and the surrounding environment. Film or simple instrumentation may be used to quantitate the chemiluminescent signal that is produced as a steady glow arising from the reaction kinetics of the system.

1,2-Dioxetane Chemiluminescent Substrates

Tropix® 1,2-dioxetane chemiluminescent substrates enable extremely sensitive detection of biomolecules by producing visible light that is detected with film or instrumentation. Applied Biosystems offers several different enzyme-activated substrates, including CDP-*Star*® and CSPD® substrates for alkaline phosphatase; Galacton,® Galacton-Plus,® and Galacton-*Star*® substrates for β-galactosidase; Glucuron® substrate for β-glucuronidase; Glucon™ substrate for β-glucosidase; and NA-*Star*® substrate for neuraminidase. Use of these reagents in immunoassays, enzyme assays, reporter gene assays, membrane-based protein detection, and nucleic acid detection on membranes or in tube or microplate assay formats offers substantial benefits compared to colorimetric, fluorescent, or isotopic detection.

Advantages of 1,2-Dioxetane Substrates

Applied Biosystems offers a wide selection of 1,2-dioxetane enzyme substrates that meet the challenging demands of a broad variety of applications. 1,2-Dioxetane substrates are non-isotopic and provide high intensity signal, low background, high sensitivity, wide dynamic range, rapid results, and are compatible with multiple assay formats under physiologically relevant conditions. The high quality, purity, and lot-to-lot consistency of Tropix substrates enable excellent reproducibility. In membrane-based assays, multiple film exposures can be acquired with standard X-ray film for over 24 hours following substrate addition.

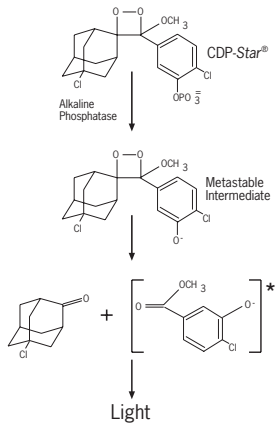

Figure 1. Light Emission Mechanism of CDP-*Star*® Substrate.

Instrumentation for Chemiluminescent Signal Detection

Luminescent signal quantitation with microplate or tube solution-based assays is performed with a luminometer, which measures light being emitted from a sample with either a PMT-based or photodiode detector, or with a CCD camera detector. Commercial luminometers include dedicated (single-mode) luminescence detection platform, or multi-mode platforms that are equipped for luminescence detection. Luminescence can also be quantitated with a scintillation counter, with the appropriate counting mode selected, or with a fluorometer, without the presence of filters that would absorb light emission. Detection with non-luminometer instrumentation does not provide optimal sensitivity, as these instruments are frequently not light-tight, and are not optimized for luminescence detection. For some substrates and assays, automatic injection capabilities are required when the kinetics of light emission are very rapid.

Signal detection with membrane-based assays, including Western blotting and Southern blotting applications, can be performed with X-ray film, photographic film, or with CCD camera-based imaging platforms. Phosphorimaging platforms can be used if the appropriate screens, requiring sensitivity to visible light emission, are available.

Microplates

Luminescence assays in a microplate format are ideally performed in opaque white microplates, including 96-, 384- or 1536-well format, depending on assay design and capability, and on instrumentation capability. Black microplates can be used, but a significant absorption of the emitted light can reduce sensitivity. For cell-based assays performed in the culture plate, white plates with a clear bottom can be utilized, permitting microscopic observation of cells prior to start of assay. With these plates, a white backing sheet is applied to the bottom of the plate prior to reading, to prevent absorption of light by the black plate platform. The continuous clear plate bottom can introduce well-well signal crosstalk, so wells with anticipated large signal differences should not be adjacent. Clear microplates are not appropriate, as well-to-well crosstalk will obscure results.

Applications

Alkaline Phosphatase Substrates

CDP-*Star*® and CSPD® substrates are used in both solution and membrane-based detection of alkaline phosphatase or alkaline phosphatase conjugates as superior alternatives to radioisotopic, colorimetric, or fluorimetric methods. For immunoassays and DNA probe assays, 1,2-dioxetane substrates improve the sensitivity [1,2,5,8,9,10,11] and generate results faster than radioimmunoassays and colorimetric detection methods. Chemiluminescent

Introduction, continued

detection of biomolecules labeled directly or indirectly with alkaline phosphatase (AP) is performed on membranes for Southern, Northern, and Western blotting, and DNA sequencing [3,4,9]. These substrates are also ideally suited for the detection of placental alkaline phosphatase (PLAP) and secreted placental alkaline phosphatase (SEAP) in reporter gene assays [6,12]. 1,2-Dioxetane substrates for alkaline phosphatase are used widely in immunoassay platforms for analytes, in identification of small molecules and viral nucleic acid detection. Additional potential applications include pasteurization monitoring, alkaline or acid phosphatase biomarker detection (stem cells, tumor markers) and sensitive quantitation of purified protein phosphatase activity [13].

β-Galactosidase Substrates

Galacton®, Galacton-Plus®, and Galacton-*Star*® substrates are widely utilized and have become the gold standard for sensitive quantitation of β-galactosidase in reporter gene assays in both mammalian and yeast cells. The chemiluminescent assay for β-galactosidase exhibits over three orders of magnitude greater sensitivity than colorimetric assays. In addition to reporter gene assays, 1,2-dioxetane substrates for β-galactosidase are used for β-galactosidase enzyme complementation assays. β-galactosidase enzyme complementation has a wide variety of applications, including both in vitro detection of biomolecules and cell-based assay systems that monitor cellular functions, such as intracellular protein-protein interactions, receptor activation, protein translocation, and cell fusion. Additional potential applications for chemiluminescent Galacton substrates include measurement of β-galactosidase activity in bacteria, for either gene expression monitoring or direct coliform detection, and immunoassay detection with β-galactosidase-labeled detection reagents.

β-Glucuronidase Substrate

Glucuron® substrate is a highly sensitive substrate for quantitating β-glucuronidase in reporter gene assays in plants or mammalian cells using the bacterial β-glucuronidase (GUS) gene. Chemiluminescent detection with Glucuron substrate exhibits greater sensitivity compared to fluorescence detection. Reporter gene assays employing Glucuron substrate are simple and convenient to perform. Additional potential applications include specific microbe detection and quantitation of mammalian β-glucuronidase. Please inquire regarding availability.

β-Glucosidase Substrate

Glucon™ substrate is utilized for highly sensitive detection of β-glucosidase. The use of Glucon substrate provides researchers with another quantitative tool for the emerging widespread use of glycosidic enzymes in environmental and biomedical testing, clinical evaluation, toxicology, and pharmaceutical screening. Please inquire regarding availability.

Neuraminidase Substrate

NA-*Star*® substrate was designed for highly sensitive chemiluminescent detection of viral neuraminidase. NA-*Star* substrate is a sensitive chemiluminescent replacement for fluorescent neuraminidase substrates, and has been used widely for global monitoring of influenza strains for resistance to neuraminidase inhibitors.

Comparison of Chemiluminescent Systems and Substrates

An important feature of Tropix® alkaline phosphatase substrates is the long-lived signal, especially on membranes. The chemiluminescent signal from CSPD® and CDP-*Star*® substrate may persist for up to several days on nylon membrane[7]. Since film exposure times range from seconds to several hours, multiple images may be acquired. Varying the film exposure time enables the user to optimize signal-to-noise. Other chemiluminescent systems, such as enhanced luminol, generate shorter-lived signals, making multiple film exposures difficult.

In solution assays (such as an immunoassay), the kinetics of CSPD and CDP-*Star* substrates are similar. Both substrates exhibit peak light emission within 30 minutes after adding substrate solution to a reaction well. Once the maximum signal is reached, it will be maintained as long as substrate is available (at least 60-90 minutes, depending on the amount of alkaline phosphatase present).

Membrane-based Detection Assays

CDP-*Star*® chemiluminescent substrate combines high intensity with rapid kinetics of light emission. This feature, coupled with up to five-fold higher signal intensity compared to CSPD substrate, makes CDP-*Star* substrate the ideal choice when rapid imaging exposures are needed. CDP-*Star* Substrate is used with Nitro-Block-II™ enhancer for nitrocellulose membranes. (see Chemiluminescence Enhancers, page 7).

Solution-based Detection Assays

CDP-*Star*® substrate also produces a higher signal in solution assays. CDP-*Star* substrate with either Sapphire-II™ or Emerald-II™ enhancer produces a signal that is nearly five-fold higher than the signal produced by CSPD® substrate with enhancer. For five-fold maximum sensitivity, CDP-*Star* Substrate solutions will provide the highest signal intensity and the greatest sensitivity.

REFERENCES (For complete reference lists, please see system-specific reference lists.)

1. Albrecht S, Ehle H, Schollberg K, Bublitz R, Horn A (1991) Chemiluminescent enzyme immunoassay of human growth hormone based on adamantly dioxetane phenyl phosphate substrate, *Bioluminescence and Chemiluminescence: Current Status*, p 115-118.
2. Bronstein I, Edwards B, Voyta JC (1989) 1,2-dioxetanes: novel chemiluminescent enzyme substrates: Applications to immunoassays, *J. Biolum. Chemilum.* **4:**99-111.
3. Bronstein I, Voyta JC, Lazzari KG, Murphy OJ, Edwards B, Kricka LJ (1990) Rapid and sensitive detection of DNA in Southern blots with chemiluminescence, *Bio Techniques* **8:**310-313.
4. Bronstein I, Voyta JC, Murphy OJ, Bresnick L, Kricka LJ (1992) Improved chemiluminescent western blotting procedure, *BioTechniques* **12:**748-753.
5. Bronstein I, Voyta JC, Thorpe GH, Kricka LJ, Armstrong G (1989) Chemiluminescent assay of alkaline phosphatase applied in an ultrasensitive enzyme immunoassay of thyrotropin, *Clin. Chem.* **35:**1 441-1446.
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12. O'Connor KL, Culp LA. (1994) Quantitation of two histochemical markers in the same extract using chemiluminescent substrates, *BioTechniques* **17:**502-509.
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CDP-*Star*® and CSPD® Substrates

Alkaline phosphatase chemiluminescent substrates

Description

CDP-*Star*® and CSPD® chemiluminescent substrates for alkaline phosphatase enable light-based detection of alkaline phosphatase and alkaline phosphatase-labeled molecules with unparalleled sensitivity, speed and ease. Chemiluminescent detection with 1,2-dioxetane substrates for alkaline phosphatase offers a highly sensitive alternative to fluorescent, isotopic, and colorimetric detection methods.

Membrane-based Applications

Tropix® chemiluminescent substrates exhibit high sensitivity detection of biomolecules labeled either directly or indirectly with alkaline phosphatase (AP) in membrane-based applications such as Southern, Northern, and Western blotting. On membranes, the faster emission kinetics and higher light intensity of CDP-*Star* substrate enable exposure times up to 10-fold shorter than CSPD substrate. Exposure times range from 1 second to 15 minutes with CDP-*Star* substrate and from 2 minutes to 2 hours with CSPD substrate with standard X-ray film. CDP-*Star* is ideal when quick film exposures are desired and in applications that require long film exposures, such as single copy gene detection by Southern analysis. Note: With nitrocellulose membrane, we recommend the use of CDP-*Star* substrate with Nitro-Block-II™ (see page 8 for substrate formulations with Nitro-Block and Nitro-Block-II).

Solution-based Applications

CDP-*Star* and CSPD substrates (Figure 2) are also used in solution-based assays such as immunoassays, nucleic acid probe assays, and direct enzyme assays. They are ideally suited for the detection of placental alkaline phosphatase (PLAP) and secreted placental alkaline phosphatase (SEAP) in reporter gene assays [1, 2]. They are used widely in immunoassay platforms for analytes identification, small molecules and viral nucleic acid detection. Maximum light levels are reached at approximately 30 minutes and glow emission persists for several hours. Emission kinetics are similar for CSPD and CDP-*Star* substrates in solution. Additional potential applications include pasteurization monitoring, alkaline or acid phosphatase biomarker detection (stem cells, tumor markers) and sensitive quantitation of purified protein phosphatase activity [3].

Product Configuration

CDP-*Star* and CSPD substrates are available as concentrates or as Ready-to-Use solutions, with or without luminescence enhancers. CDP-*Star* and CSPD Substrate concentrates are supplied at 5 or 25 mM (respectively) in aqueous buffer. The recommended working concentration for these substrates is 0.4 mM for solution-based assays and 0.25 mM for membrane-based assays. Ready-to-Use formulations of 0.25 mM substrate for membrane-based applications are listed below. Ready-to-Use substrate/enhancer formulations for solution-based assays are listed below and described on page 7. The use of enhancers is necessary in solution assays for optimal light output and sensitivity (see page 7 for enhancers and Ready-to-Use substrate/enhancer formulations).

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
CDP-*Star*® Chemiluminescent Substrate for Alkaline Phosphatase		
5 mM Concentrate	1.0 mL	T2304
5 mM Concentrate	2.0 mL	T2305
5 mM Concentrate	5.0 mL	T2306
5 mM Concentrate	10 mL	T2307
5 mM Concentrate	20 mL	T2308
5 mM Concentrate	50 mL	T2309
5 mM Concentrate	200 mL	T2310
Ready-to-Use (0.25 mM)	50 mL	T2145
Ready-to-Use (0.25 mM)	100 mL	T2146
Ready-to-Use (0.25 mM)	250 mL	T2147
Ready-to-Use (0.25 mM) with Nitro-Block-II™ Enhancer	100 mL	T2218
Ready-to-Use (0.4 mM) with Sapphire-II™ Enhancer	100 mL	T2214
Ready-to-Use (0.4 mM) with Emerald-II™ Enhancer	100 mL	T2216
Larger sizes of CDP-*Star*® are available upon request.		

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APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
CSPD® Chemiluminescent Substrate for Alkaline Phosphatase		
25 mM Concentrate	25 mL	T2044
25 mM Concentrate	0.5 mL	T2040
25 mM Concentrate	1.0 mL	T2098
25 mM Concentrate	2.5 mL	T2041
25 mM Concentrate	5.0 mL	T2042
25 mM Concentrate	10 mL	T2043
Ready-to-Use (0.25 mM)	50 mL	T2141
Ready-to-Use (0.25 mM)	100 mL	T2142
Ready-to-Use (0.25 mM)	250 mL	T2143
Ready-to-Use (0.25 mM)) with Nitro-Block™ Enhancer	100 mL	T2217
Ready-to-Use (0.4 mM) with Sapphire-II™ Enhancer	100 mL	T2210
Ready-to-Use (0.4 mM) with Emerald-II™ Enhancer	100 mL	T2212
Larger sizes of CSPD® are available upon request.		

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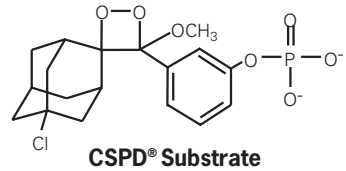
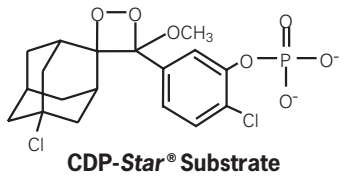


CSPD® Substrate

Panel A.



CDP-*Star*® Substrate

Panel B.

Figure 2. Chemical Structures of CSPD® (Panel A) and CDP-*Star*® (Panel B) Substrates.

REFERENCES

1. Bronstein I, Fortin JJ, Voyta JC, Juo RR, Edwards B, Olesen CE, Lijam N, Kricka LJ (1994) Chemiluminescent reporter gene assays: Sensitive detection of the GUS and SEAP gene products, *BioTechniques* **17:**172-178.
2. O'Connor KL, Culp LA. (1994) Quantitation of two histochemical markers in the same extract using chemiluminescent substrates, *BioTechniques* **17:**502-509.
3. Zhao S, Zhu Q, Somerville RL (2000) The σ(70) transcription factor TyrR has zinc-stimulated phosphatase activity that is inhibited by ATP and tyrosine. *J Bacteriol.* **182(4):**1053-1061.

Galacton®, Galacton-Plus® and Galacton-*Star*® Substrates

β-Galactosidase chemiluminescent substrates

Description

Galacton®, Galacton-Plus®, and Galacton-*Star*® chemiluminescent substrates (Figure 3) for β-galactosidase provide chemiluminescent detection of β-galactosidase and β-galactosidase-labeled molecules. These substrates offer significant sensitivity improvement over colorimetric and fluorescent detection methods.

Applications

Galacton,® Galacton-Plus,® and Galacton-*Star*® substrates have been very widely used for reporter gene assays in many different organisms, including mammals, yeast (including two hybrid assays), bacteria, protozoans, fish cells, and frog oocytes. Galacton-*Star* substrate has been employed in β-galactosidase enzyme complementation technology, which provides a novel cell-based assay capability for intracellular monitoring of protein-protein interactions, cell fusion assays, and intracellular protein translocation. Additional applications include detection of β-galactosidase conjugates in immunoassay and immunodetection formats, and detection of endogenous bacterial β-galactosidase for coliform enumeration.

Substrate Selection

Galacton substrate is a first-generation substrate that enables highly sensitive detection of β-galactosidase in reporter gene assays and direct enzyme assays. Galacton-Plus® chemiluminescent substrate for β-galactosidase provides prolonged emission kinetics and superior sensitivity compared to Galacton substrate. Light emission is maintained for several minutes with Galacton-Plus substrate, which is necessary when light signal is measured in a microplate luminometer without automatic injection capabilities. Figure 4 shows the kinetics of detection of purified β-galactosidase with Galacton substrate and Galacton-Plus substrate. Galacton-*Star* chemiluminescent substrate enables detection in a single-step reaction format. Light emission with Galacton-*Star* substrate typically reaches maximum in 60 minutes and exhibits glow kinetics for nearly one hour (Figure 5). Galacton-*Star* substrate provides the most facile detection protocol, requiring only a single reagent addition to sample, and in addition, provides the highest sensitivity detection of β-galactosidase. For new users, the Galacton-*Star* substrate, included in the Galacto-*Star* and Gal-Screen® reporter gene assay systems, is recommended for all applications. Enhancers and/or accelerators are required for optimum performance with each of these substrates, and are provided as kit components with each of the β-galactosidase reporter gene assay systems (see page 12 for Gal-Screen system; page 14 for Galacto-*Star* system; page 16 for Galacto-Light™ system and Galacto-Light Plus™ system; page 21 for Dual-Light® system).

Product Configuration

Galacton and Galacton-Plus substrates are supplied as 100X concentrates. Galacton substrate is also available as a component of the Galacto-Light system. Galacton-Plus substrate is also available in the Dual-Light and Galacto-Light Plus systems. Galacton-*Star* substrate is supplied as a 10 mM concentrate, and is diluted 50-fold for use. Galacton-*Star* substrate is also available in the Galacto-*Star* and Gal-Screen systems. For reporter gene assays, we recommend purchase of the complete systems, so that all necessary reagents and protocol are obtained.

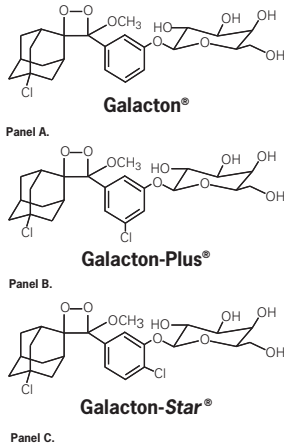


Figure 3. Chemical Structures of β-Galactosidase Substrates.

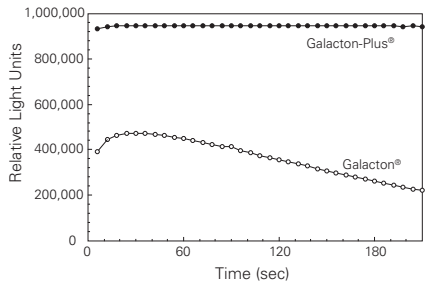


Figure 4. Detection of β-Galactosidase with Galacton® and Galacton-Plus® Substrates.

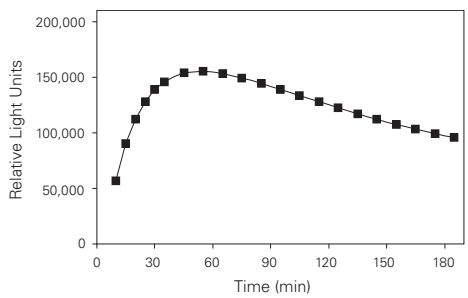


Figure 5. Detection of β-Galactosidase with Galacton-Star® Substrate.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Galacton® Chemiluminescent Substrate for β-Galactosidase		
100X Concentrate	2 mL	T2094
100X Concentrate	4 mL	T2121
100X Concentrate	10 mL	T2095
Galacton-Plus® Chemiluminescent Substrate for β-Galactosidase		
100X Concentrate	2 mL	T2118
100X Concentrate	4 mL	T2119
100X Concentrate	10 mL	T2120
Galacton-Star® Chemiluminescent Substrate for β-Galactosidase		
10 mM Concentrate	2 mL	T2264
10 mM Concentrate	4 mL	T2265
10 mM Concentrate	10 mL	T2266

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Glucuron® Substrate

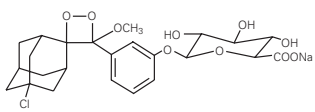
β-Glucuronidase chemiluminescent substrate

Description

Glucuron® chemiluminescent substrate enables chemiluminescent detection of β-glucuronidase (GUS). Glucuron substrate is a highly sensitive alternative to colorimetric and fluorescent detection methods. The superior sensitivity achieved with Glucuron substrate makes it the ultimate choice for GUS reporter gene assays in plant or mammalian cells.

Applications

Quantitation of glycosidic enzymes has wide-spread application, including reporter gene assays, environmental testing, biomedical research, toxicology, and pharmaceutical screening. Glucuron substrate can be employed for sensitive chemiluminescent detection in these varied/various applications, and has primarily been used for GUS reporter gene assays [1]. Measurement of GUS activity has been used in a novel bioassay with *E. coli* to evaluate toxicity of metal ions in environmental samples [4]. Additional potential applications include specific microbe detection and detection of mammalian β-glucuronidase. Mammalian β-glucuronidase activity has been measured in assays for mast cell degranulation [2] and to monitor periodontal disease [3].



Glucuron®

Figure 6. Chemical Structure of Glucuron® Substrate.

REFERENCES

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4. Mariscal A, Garcia A, Carnero M, Gomez E, Fernandez-Crehuet J (1994) New toxicity determination method that uses fluorescent assay of Escherichia coli, *Biotechniques* **16:**888-892.

Product Configuration

Glucuron substrate is supplied as a 100X concentrate. Please inquire regarding availability.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Glucuron® Substrate		
Tropixcustom (GL005) 100X Concentrate	0.5 mL	Tropixcustom
Tropixcustom (GL010) 100X Concentrate	1.0 mL	Tropixcustom
Tropixcustom (GL025) 100X Concentrate	2.5 mL	Tropixcustom

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Glucon™ Substrate

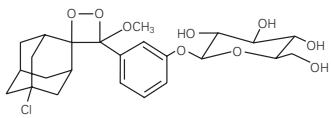
β-Glucosidase chemiluminescent substrate

Description

Glucon™ chemiluminescent substrate enables chemiluminescent detection of β-glucosidase. Glucon, is a highly sensitive alternative to colorimetric and fluorescent detection methods.

Applications

β-Glucosidase has been used in conjunction with β-glucuronidase in a physiologic test for the rapid identification and differentiation of enterococci and streptococci [2]. Vectors containing the sequence for β-glucosidase have been developed [1]. In addition, thermostable β-glucosidase has been cloned and isolated [1] and could provide an extremely sensitive, robust reporter assay system that will eliminate background from endogenous enzyme activity. Glucon™ substrate can also be used for detection of mammalian β-glucosidase activity.



Glucon™

Figure 7. Chemical Structure of Glucon™ Substrate.

REFERENCES

1. Gabelsberger J, Liebl W, Schleifer K (1993) Purification and properties of recombinant β-glucosidase of the hyperthermophilic bacterium Thermotoga maritime, *Appl Microbiol Technol* **40:**44-52.
2. Kirby R, Ruoff K (1995) Cost-effective, clinically relevant method for rapid identification of beta-hemolytic streptococci and enterococci, *J Clin Microbiol* **33:**1154-1157.

Product Configuration

Glucon substrate is supplied as a 10 mM concentrate. The suggested working concentration for solution-based assays is 0.1 mM. Please inquire regarding availability.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Glucon™ Substrate		
Tropixcustom	10 mM concentrate	Tropixcustom

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NA-*Star*® Substrate

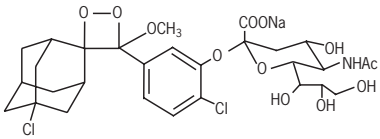
Neuraminidase chemiluminescent substrate

Description

NA-*Star*® chemiluminescent substrate enables sensitive detection of neuraminidase (NA) activity. This substrate is a highly sensitive replacement for the widely used fluorogenic substrate, methylumbelliferyl N-acetylneuraminic acid (MUNANA). NA-*Star* substrate has been incorporated into the NA-*Star* Influenza Neuraminidase Inhibitor Resistance Detection Kit. The NA-*Star* Influenza Neuraminidase Inhibitor Resistance Detection Kit includes everything needed to quantitate neuraminidase activity and neuraminidase inhibitor resistance in avian, equine, human (types A and B), and porcine influenza viruses. The kit's fast and easy protocol and convenient 96-well plate format make it ideal for monitoring influenza virus neuraminidase inhibitor resistance, as well as high-throughput inhibitor compound screening.

Applications

NA-*Star* chemiluminescent substrate has applications in viral research for the detection and characterization of viral neuraminidases. NA-*Star* substrate has been applied to the detection of influenza virus neuraminidase activity in clinical isolates providing up to 60-fold higher sensitivity than with the fluorescence assay [1]. The chemiluminescent-based detection technology provides a wide dynamic range—greater than four orders of magnitude of neuraminidase concentration (two orders of magnitude greater than fluorescent MUNANA-based assays), enabling accurate quantitation of neuraminidase inhibitor resistance levels over a broad range of virus concentration and neuraminidase activity without having to test multiple virus dilutions. To date, this substrate has been used primarily for global screening of flu strains for neuraminidase inhibitor sensitivity. Additional applications include high throughput screening for identification of new neuraminidase inhibitor anti-viral therapeutics, detection of neuraminidase in other organisms, including bacteria.



NA-*Star*®

Figure 8. Chemical Structure of NA-*Star*® Substrate.

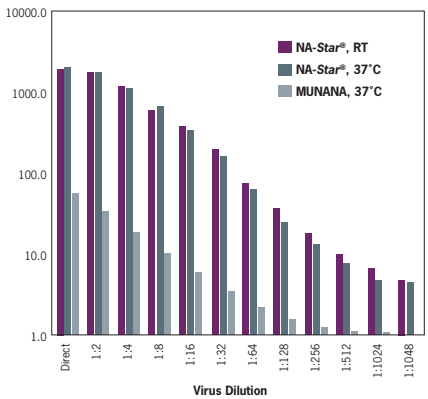


Figure 9. Sensitivity Comparison of Chemiluminescent Assay Detection with NA-*Star*® Substrate to Fluorescent Assay Detection with MUNANA Substrate. Dilutions of Influenza Type B (ATCC VR-1535) virus culture supernatant (cultured on MDCK cells) were assayed at different temperatures and Signal/Noise (S/N) ratio calculated using uninfected MDCK cell supernatant (Noise). The lower limit of detection (S/N = 2) is at least 30-fold lower with the chemiluminescent NA-*Star* assay, and S/N is approx. 50-fold higher with NA-*Star* assay. The dynamic range of detection with the NA-*Star* assay with virus samples is three orders of magnitude.

The fast and easy protocol enables you to perform assays in less than 1.5 hours. Simply incubate your virus samples with dilutions of neuraminidase inhibitor, add NA-*Star* chemiluminescent substrate, incubate, and then inject/add the accelerator solution, which triggers light emission from the reaction product. Light signal is measured with a luminometer, including multi-mode instruments that include a luminometer mode. For best results, use a luminometer with an automatic injector to add the accelerator solution, although a multichannel pipettor can be used if plate is read immediately.

Product Configuration

The NA-*Star* Influenza Neuraminidase Inhibitor Resistance Detection Kit includes NA-*Star* chemiluminescent substrate for neuraminidase, all necessary assay reagents, and microplates everything needed for fast, accurate quantitation of neuraminidase inhibitor resistance in influenza virus isolates.

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9. Neuraminidase Inhibitor Susceptibility Network (2005) Evolution of HSNI avian influenza viruses in Asia, *Emerging Infectious Diseases* **11(10):**1515-1521.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit	960 assay wells + 10 x 96-well microplates	4374422
NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Reagent Set	960 assay wells	4374348
NA-*Star*® Detection Microplates	10 x 96-well microplates	4374349
NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit Protocol	1 protocol	4375714

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Sapphire™, Sapphire-II™, Emerald™, Emerald-II™ and Ruby™ Enhancers

Luminescence enhancers for solution-based assays

Chemiluminescent Enhancement

Applied Biosystems has developed macromolecular signal-enhancement technology for use with 1,2-dioxetane enzyme substrates in solution-based assays. Aqueous environments reduce the chemiluminescent signal intensity by water-induced quenching. Addition of Tropix® luminescence enhancers increases the emission efficiency of light production by partitioning the water away from the site of signal production. Tropix enhancers such as Sapphire™, Emerald™, Ruby™, Sapphire-II™, and Emerald-II™ enhancers are essential components of solution-based assays. Tropix enhancers provide signal enhancement with minimal delay of light-emission kinetics. For example, adding Sapphire enhancer to chemiluminescent substrate in 0.1 M diethanolamine (DEA) (pH 10) increases the chemiluminescent half-time to plateau (T1/2) by only 15-20% [1].

Choosing an Enhancer

Sapphire™, Emerald™, Ruby™, Sapphire-II™, or Emerald-II™ Enhancers

Tropix enhancers shift the wavelength of light emission. Sapphire and Sapphire-II enhancers slightly shift the light emission maximum from 475 nm observed for the dioxetane alone to 461 nm. Emerald and Emerald-II enhancers shift the light emission maximum to 542 nm, while Ruby™ enhancer shifts the light emission maximum to 620 nm. Although the signal intensity obtained with Emerald and Emerald-II enhancers is much greater compared to other enhancers, use of Sapphire and Sapphire-II enhancers produces a wider dynamic range since photodetector saturation is less likely to occur. The Emerald or Emerald-II enhancer is the optimum choice for applications requiring maximum signal intensity. Applied Biosystems offers an enhancer variety pack to help match the best enhancer for your application and instrumentation.

Applications

Tropix enhancers are essential components in immunoassays, nucleic acid hybridization assays performed in a microplate or similar format, reporter gene assays, and enzyme assays because of improved signal-to-noise performance. Enhancers are included in the ELISA-Light™ system (page 32) and reporter gene assay system reagents (pages 10-24).

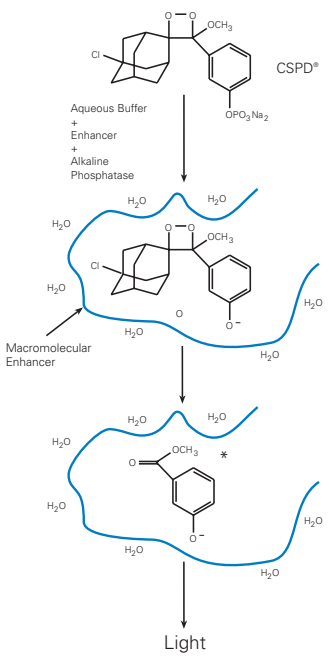

Figure 10. Chemiluminescence Enhancement.

Enhancer Variety Pack

Sample Sizes of Sapphire™, Emerald , Sapphire-II™ and Emerald-II™ Enhancers

Optimizing signal intensity with the appropriate enhancer can be easily accomplished with a selection of enhancers. The Enhancer Variety Pack contains a sample of Sapphire, Sapphire-II, Emerald, and Emerald-II enhancers for solution-based applications in convenient 5 mL sizes.

Ready-to-Use Substrate/Enhancer Formulations

CSPD® Ready-to-Use Formulations (with Sapphire-II™ or Emerald-II™ Enhancers), CDP-Star® Ready-to-Use Formulations (with Sapphire-II™ or Emerald-II™ Enhancers)

Ready-to-Use substrate/enhancer formulations are supplied at 0.4 mM substrate and 1X enhancer in a stable buffer system, eliminating the need for substrate dilution buffer preparation and substrate and enhancer dilution. Ready-to-Use formulations simplify procedures for immunoassays, nucleic acid hybridization assays in microplate formats, enzyme assays, and other solution-based assays.

Enhancer	Maximum Light Emission	S/N
Sapphire-II™ Enhancer	461 nm	HIGH
Sapphire™ Enhancer	461 nm	MODERATE
Comments: Provides optimum signal-to-noise ratio (S/N) and best dynamic range for highly sensitive luminometer measurement		
Emerald-II™ Enhancer	542 nm	HIGH
Emerald™ Enhancer	542 nm	MODERATE
Comments: Provides highest signal intensity for assays performed in black microplates; for camera imaging systems requiring high intensity signal		
Ruby™ Enhancer	620 nm	MODERATE
Comments: Provides wavelength shift that may be compatible with some solid state detectors		

Figure 11. Enhancer Comparison for Solution-based Assays.

REFERENCES

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APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Sapphire™ 10X Concentrate	25 mL	T2011
Sapphire-II™ 10X Concentrate	25 mL	T2113
Emerald™ 10X Concentrate	25 mL	T2002
Emerald-II™ 10X Concentrate	25 mL	T2115
Ruby™ 10X Concentrate	25 mL	Tropixcustom
Enhancer Variety Pack	4 x 5 mL	Tropixcustom
Ready-to-Use Substrate/Enhancer Formulations		
CSPD® Ready-to-Use with Sapphire-II™	100 mL	T2210
CSPD® Ready-to-Use with Emerald-II™	100 mL	T2212
CDP-*Star*® Ready-to-Use with Sapphire-II™	100 mL	T2214
CDP-*Star*® Ready-to-Use with Emerald-II™	100 mL	T2216
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Nitro-Block™ and Nitro-Block-II™

Luminescence enhancers for membrane-based assays

Chemiluminescent Enhancement on Membranes

Membranes commonly used in nucleic acid and protein detection applications include nylon, nitrocellulose, and PVDF (polyvinylidene fluoride). The signal intensity generated from 1,2-dioxetane substrates varies on each of the membranes.

Nylon Membranes

Nylon membranes are essentially "self-enhancing" since the surface contains hydrophobic micro-domains compatible with the chemiluminescence process. The use of enhancer on these membranes is not recommended.

Nitrocellulose and PVDF Membranes

Nitrocellulose membranes provide an inefficient environment for chemiluminescence, resulting in very low signal intensity. Tropix® Nitro-Block™ and Nitro-Block-II™ membrane enhancers increase signal intensity on both nitrocellulose and PVDF membranes [1]. Nitro-Block and Nitro-Block-II enhancers generate a hydrophobic environment on the membrane surface that increases the intensity of chemiluminescence. The effects of Nitro-Block enhancer treatment on nitrocellulose and PVDF membranes are shown (Figure 12). Without Nitro-Block enhancer, the signal on nitrocellulose membranes is weak, making detection either impossible or requiring extremely long exposure times. With Nitro-Block enhancer, short exposures of 10 to 45 minutes are achieved. While PVDF membranes do not require the use of Nitro-Block enhancer, exposure times are tenfold faster with Nitro-Block enhancer. This permits very short exposure times on PVDF, ranging from 15 seconds to 15 minutes for Western blots.

Nitro-Block™ Enhancer Compared to Nitro-Block-II™ Enhancer

Nitro-Block-II membrane enhancer is a modified version of Nitro-Block membrane enhancer that has been developed to further optimize the performance of chemiluminescent 1,2-dioxetane substrates. Nitro-Block or Nitro-Block-II enhancers are required for use with CSPD® substrate on nitrocellulose membranes. Nitro-Block-II Enhancer is required for use with CDP-*Star*® substrate on nitrocellulose membranes. The use of Nitro-Block-II Enhancer is not necessary with CDP-*Star* substrate on PVDF membranes.

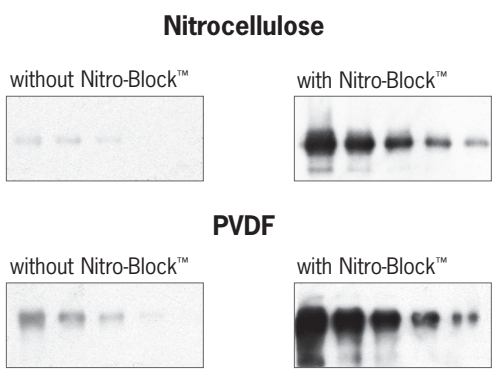

Figure 12. Chemiluminescent Signal Enhancement on Nitrocellulose and PVDF Membranes with Nitro-Block™ Enhancer. X-ray images of Western blots detected using CSPD® substrate.

Ready-to-Use Substrate/Enhancer Formulations

CSPD® Ready-to-Use Substrate with Nitro-Block™, CDP-*Star*® Ready-to-Use Substrate with Nitro-Block-II™

Ready-to-Use formulations for membrane-based applications are supplied with enhancer pre-mixed with substrate for convenience and ease of use. The shelf-life for all the Ready-to-Use formulations is one year at 4°C. Ready-to-Use formulations with Nitro-Block and Nitro-Block-II Enhancer are intended for use with nitrocellulose membranes to increase light intensity. Substrate/enhancer formulations for membrane-based applications are supplied at 0.25 mM substrate and 1X enhancer concentrations. Note: prior to using Ready-to-Use formulations, a high pH wash of the membrane with assay buffer is recommended.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Nitro-Block™ 20X Concentrate	20 mL	T2026
Nitro-Block-II™ 20X Concentrate	20 mL	T2184
Ready-to-Use Substrate/Enhancer Formulations		
CSPD® Ready-to-Use with Nitro-Block™	100 mL	T2217
CDP-*Star*® Ready-to-Use with Nitro-Block-II™	100 mL	T2218

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REFERENCES

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Substrate	Enhancer	Performance
Nitrocellulose		
CSPD® Substrate	Nitro-Block™ or Nitro-Block-II™	
CDP-*Star*® Substrate	Nitro-Block-II™	Optimal
PVDF		
CSPD® Substrate	Nitro-Block™ or Nitro-Block-II™	
CDP-*Star*® Substrate	—	Optimal
Nylon		
CSPD® or CDP-*Star*®	—	Optimal

Figure 13. Enhancer Comparison for Membrane-based Assays.

2 Reporter Gene Assays and Reagents

Introduction

Reporter Gene Assays

Reporter gene assays are invaluable for studying regulation of gene expression, both by cis-acting factors (gene regulatory elements) or trans-acting factors (transcription factors or exogenous regulators). In these assays, the reporter gene acts as a surrogate for the coding region of the gene under study. The reporter gene construct contains one or more gene regulatory elements being analyzed, the structural sequence of the reporter gene, and the sequences required for the formation of functional mRNA. Upon introduction of the reporter construct into cells, expression levels of the reporter gene are monitored through a direct assay of the reporter protein's enzymatic activity. The sensitivity of each reporter gene assay is a function of several factors including detection method, reporter mRNA and protein turnover, and endogenous (background) levels of the reporter activity. Both protein turnover and levels of endogenous background vary with each reporter protein and the cell line used. Commonly used detection techniques utilize isotopic, colorimetric, fluorometric, or luminescent enzyme substrates and immunoassay-based procedures with isotopic, colorimetric, or chemiluminescent end points.

Common Reporter Genes

Below is a list of the most common reporter genes, detection methods for the reporter protein, and corresponding detection limits. β-Galactosidase and luciferase are among the most widely used reporter genes to date. β-Galactosidase is often used in conjunction with other reporter genes to normalize transfection efficiency. β-Galactosidase is traditionally detected with the colorimetric substrate o-nitrophenyl β-D-galactopyranoside (ONPG) [1]. As indicated in table, this colorimetric assay is less insensitive compared to many other reporter gene assays. With Tropix™ 1,2-dioxetane chemiluminescent substrates for β-galactosidase, the sensitivity is increased dramatically [3,4].

The assay for chloramphenicol acetyl transferase (CAT) exhibits only moderate sensitivity, suffers from a narrow dynamic range, and usually incorporates radioisotopes. This reporter gene assay method is now only infrequently used. Assays with limited dynamic range, such as CAT, require testing of several sample dilutions to verify that sample values are within the linear range.

Secreted placental alkaline phosphatase (SEAP) is secreted by cells directly into the culture media, and can be assayed simply by taking samples of cell culture media. Secreted reporter proteins enable non-destructive assay of cell culture medium, preserving cells for additional assays and enabling time-course monitoring of gene expression. SEAP is detected with both colorimetric and chemiluminescent substrates. Phospha-Light™ Assay System, the chemiluminescent SEAP reporter gene assay, exhibits remarkable sensitivity and ease of use. Human growth hormone (hGH), another secreted reporter protein, is detected using a radioimmunoassay (RIA) procedure, has disadvantages associated with the use of radioisotopes, and exhibits only a moderate detection limit.

Luciferase has become increasingly popular as a reporter gene, especially for co-transfection experiments where it is important to normalize transfection efficiency. As with the Tropix chemiluminescent reporter gene assays, the bioluminescent luciferase assay offers high sensitivity and a simple assay procedure. The high level of sensitivity attained with this assay is partly due to the lack of luciferase activity in most cell types (see pages 18-22 for luciferase assay systems). Dual-Light® Assay System, a combined reporter gene assay system for the sequential detection of luciferase and β-galactosidase, enables the user to perform both measurements from a single aliquot of cell extract in the same reaction well or tube (see pages 21 and 22 for Dual-Light kit), minimizing experimental error.

Reporter Gene	Detection Method	Detection Limit	Advantages	Disadvantages
Chloramphenicol Acetyl Transferase (CAT)	Isotopic ELISA	$5x10^7$ molecules $1x10^9$ molecules	Widely Used No Radioactivity	Radioactive High Cost Low Dynamic Range Labor Intensive Low Dynamic Range High Cost/Assay Labor Intensive
β-Galactosidase	ONPG (Color) MUG (Fluorescence) Gal-Screen® System Galacto-*Star*™ System Galacto-Light Plus™ System and Dual-Light® System (Luminescence)	$3x10^8$ molecules $6x10^5$ molecules $3x10^3$ molecules	Widely Used High Sensitivity Wide Dynamic Range Simplicity	Poor Sensitivity Autofluorescence
Human Growth Hormone	Radioimmunoassay	$3x10^8$ molecules	Secreted into Media	Radioactivity High Cost/Assay Low Sensitivity
Luciferase	Luc-Screen® System Tropix Luciferase Assay Kit Dual-Light® System (Luminescence)	10^3-10^4 molecules	Assay Simplicity High Sensitivity Wide Dynamic Range	Protein Instability
β-Glucuronidase	MUG (Fluorescence) Glucuron® Substrate (Luminescence)	$2x10^8$ molecules $5x10^5$ molecules	Autofluorescence High Sensitivity Simplicity Wide Dynamic Range	Protein Quenching
Secreted Placental Alkaline Phosphatase	pNPP (Color) Phospha-Light™ System (Luminescence)	$1x10^8$ molecules $3x10^4$ molecules	Secreted into Media Secreted into Media High Sensitivity Wide Dynamic Range	Poor Sensitivity

Figure 14. Comparison of Reporter Gene Assays

Introduction, continued

Bacterial β-glucuronidase (GUS) is one of the most widely used reporter genes in plant genetic research. It is also used to a lesser extent in mammalian cells. In plant cells, β-glucuronidase activity is absent or present at very low levels. Although GUS is present in mammalian cells, its pH profile is dramatically different compared to the transfected bacterial form, enabling discrimination between the two [10]. GUS is commonly measured in extracts using the fluorescent substrate MUG. Glucuron® chemiluminescent substrate (as a component of the discontinued GUS-Light™ reporter gene assay system) has been used for highly sensitive detection in GUS reporter gene assays in plant [6,7,8], mammalian [4,12], yeast [11] and arthropod cells [9]. For information on running GUS reporter gene assays with Glucuron substrate, please contact Applied Biosystems Technical Support.

GENERAL REFERENCES

1. Alam J, Cook JL (1990) Reporter genes: application to the study of mammalian gene transcription, *Anal Biochem* **188:**245-254.
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B-Glucoronidase (Glucuron® substrate)

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Reporter Assay System	Reporter Enzyme(s)	Key Attributes	Substrate	Duration of Light Emission	Reagent Injection	Special Features
Gal-Screen® see page 12	β-Galactosidase	Homogeneous assay does not require removal of culture medium.	Galacton–*Star*®	60-90 min (+)	Not required	Designed for assays on cells cultured in luminometer plates for high-throughput screening. Choice of reaction buffers for lysis of mammalian or yeast and mammalian cells.
Galacto-*Star*™ see page 14	β-Galactosidase	Single step addition of substrate and enhancer	Galacton–*Star*®	1 hr (+)	Not required	Useful for high throughput applications. Choice of lysis buffer for yeast and mammalian model systems. Recommended for all new users.
Galacto-Light Plus™ see page 16	β-Galactosidase	Extended light emission and better sensitivity than Galacto-Light™	Galacton-Plus®	30-60 min	Recommended	Established system with high sensitivity.
Galacto-Light™ see page 16	β-Galactosidase	First chemiluminescent system for detection of β-galactosidase	Galacton®	Several minutes	Required	Established system with good sensitivity.
Luc-Screen® see page 18	Firefly Luciferase	Extended-glow homogeneous assay does not require removal of culture medium	Luciferin	90 min (+)	Not required	Designed for assays on cells cultured in luminometer plates for high-throughput screening.
Luciferase Assay Kit see page 20	Firefly Luciferase	Enhanced signal	Luciferin	Several minutes	Required	Lysis buffer compatible with other Tropix® reporter systems.
Dual-Light® see page 21	Firefly Luciferase, β-Galactosidase	Detection of luciferase and β-galactosidase from same cell extract	Luciferin Galacton-Plus®	5 min/30 min	Required	Two assays from one cell extract enables better precision for normalizing transfection efficiency.
Phospha-Light™ see page 23	Secreted placental alkaline phosphatase	No cell lysis required	CSPD®	1-2 hr (+)	Not required	Cells remain viable; useful for stable transfectants, time course studies, etc.

Figure 15. Selection Guide for Reporter Gene Assay Systems. Applied Biosystems offers several highly sensitive reporter gene assay systems. This chart describes the differences and relative merits of each system to help you choose the most appropriate system.

Gal-Screen® Assay System

Homogeneous β-galactosidase *reporter gene assay system for mammalian or yeast cells*

Description

The Gal-Screen® assay system combines direct cell lysis with rapid ultra-sensitive chemiluminescent detection of β-galactosidase reporter enzyme. This homogeneous assay is ideally suited for screening applications where assay automation is required. Gal-Screen system uses Galacton-*Star*® chemiluminescent substrate and Sapphire-II™ luminescence enhancer. A single reagent, providing cell lysis and chemiluminescent enzyme substrate, is added to cells in the presence of culture medium with or without phenol red. Light emission reaches maximum in 60-90 minutes and remains constant for 45–90 minutes.

The Gal-Screen system utilizes a simple protocol that can be used with either mammalian or yeast cells (Figure 18). A single assay reagent, which provides cell lysis and contains all reaction components, is prepared by adding Galacton-*Star* substrate to Reaction Buffer A or B. The assay reagent is added to an equal volume of cells in culture medium in either 96-, 384- or 1536-well microplates. After an incubation period, light emission is measured in a luminometer.

Advantages

The Gal-Screen assay provides greater sensitivity than colorimetric or fluorescent assays. With a lower limit of detection of 1 picogram in the presence of culture medium (Figure 16), this assay provides excellent sensitivity compared to other reporter systems. Colorimetric reporter gene assays cannot rival the dynamic range of the Galacton-*Star*® substrate in the Gal-Screen system. The wide dynamic range spans five orders of magnitude, from picogram to nanogram levels, enabling detection of a wide range of reporter enzyme concentration in cells. The assay protocol was developed particularly for use with automation. The Gal-Screen assay protocol is adaptable for use in 96-, 384- or 1536-well microplate formats (Figure 19), with either mammalian or yeast cells.

Applications

The Gal-Screen assay system is widely used for traditional reporter gene assays in transiently and stably-transfected mammalian cells [7, 11], including assays for studying viral infectivity and function [8, 12, 15]. It is widely used for reporter gene assays in yeast cells [3, 9, 10], including quantitative yeast two hybrid analysis [13, 14, 16]. In addition, it has been used for reporter gene assays in fish cells [6] and bacterial cells [5]. Gal-Screen assays provide highly sensitive detection for β-galactosidase complementation assays used for intracellular monitoring of protein-protein interactions [4], protein translocation [17], and receptor dimerization/activation [1, 2], including for high throughput compound screening for receptor activation [18].

Product Configuration

The Gal-Screen β-galactosidase reporter gene assay system is formatted with two alternative Reaction Buffers for lysis of mammalian or yeast cells. Reaction Buffer A is for use with mammalian cells, while Reaction Buffer B is for yeast or mammalian cells. In comparison on mammalian cells (Figure 18), Reaction Buffer A provides a faster time to peak light emission with a shorter duration of peak signal, while Reaction Buffer B requires a longer time to peak and provides a longer duration of glow light emission.

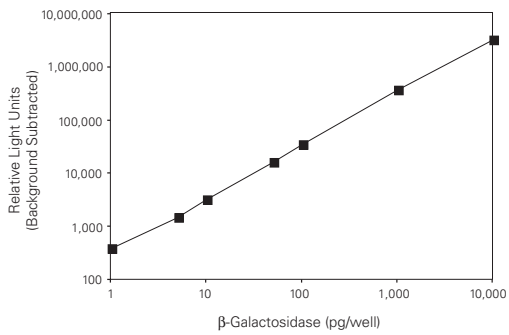

Figure 16. Sensitivity of Gal-Screen® Assay Detection. Gal-Screen® assays were performed with the indicated amount of purified β-Galactosidase diluted in PBS. Assays were measured on a microplate luminometer.

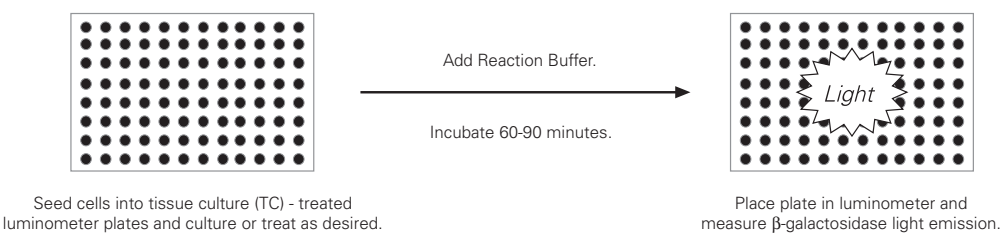

Figure 17. β-Galactosidase Reporter Gene Assay with Gal-Screen® System.

Gal-Screen® Assay System, continued

Homogeneous β-galactosidase reporter gene assay system for mammalian or yeast cells

The system is available in three sizes:

Gal-Screen® System Standard Size

T1029 – Reaction Buffer A
T1032 – Reaction Buffer B
- 0.8 mL Galacton-*Star*® Substrate
- 19.2 mL Reaction Buffer A (for mammalian cells) or 19.2 mL Reaction Buffer B (for yeast or mammalian cells)

Gal-Screen® System Large Size

T1027 – Reaction Buffer A
T1030 – Reaction Buffer B
- 4 mL Galacton-*Star*® Substrate
- 96 mL Reaction Buffer A (for mammalian cells) or 96 mL Reaction Buffer B (for yeast or mammalian cells)

Gal-Screen® System Screening Size

T1028 – Reaction Buffer A
T1031 – Reaction Buffer B
- 42 mL Galacton-*Star*® Substrate
- 1 L Reaction Buffer A (for mammalian cells) or 1 L Reaction Buffer B (for yeast or mammalian cells)

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Gal-Screen® System for Mammalian Cells	200 assays	T1029
Gal-Screen® System for Mammalian Cells	1,000 assays	T1027
Gal-Screen® System for Mammalian Cells	10,000 assays	T1028
Gal-Screen® System for Yeast or Mammalian Cells	200 assays	T1032
Gal-Screen® System for Yeast or Mammalian Cells	1,000 assays	T1030
Gal-Screen® System for Yeast or Mammalian Cells	10,000 assays	T1031

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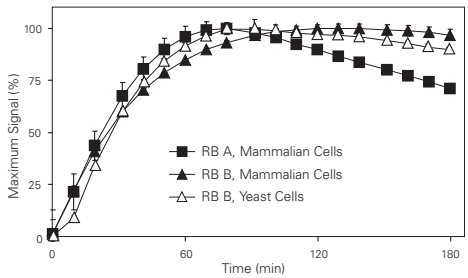

Figure 18. Kinetics of Gal-Screen® Assay. Gal-Screen® assays were performed with mammalian or yeast cells, with Reaction Buffer A and B. Assays were measured on a microplate luminometer. Data is presented as the percentage of the maximum signal.

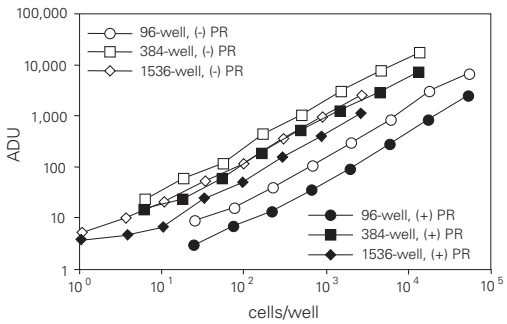

Figure 19. Gal-Screen® Assay Detection of ψ2BAG∝ Cells. Gal-Screen® assays were performed on ψ2BAG∝ cells, which constitutively express β-Galactosidase. Assays were performed in several microplate formats, in media either with or without phenol red. Measurements were performed on a CCD microplate luminometer.

β-Galactosidase (Gal-Screen® Reporter Gene Assay System; for a complete reference list, please see the Applied Biosystems website.)

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Galacto-*Star*™ Assay System

One-step β–galactosidase reporter gene assay

Description

Galacto-*Star*™ Assay System is a chemiluminescent reporter assay system designed for the rapid, and sensitive detection of β-galactosidase in cell lysates. The chemiluminescent assay exhibits over three orders of magnitude greater sensitivity compared to colorimetric β-galactosidase assays.

Galacto-*Star* assay system includes Galacton-*Star* chemiluminescent β-galactosidase substrate. Automatic reagent injection is not required. Cell lysate is mixed with Reaction Buffer containing Galacton-*Star* substrate and Sapphire-II™ enhancer. Light emission from Galacto-*Star* reaches maximum in 60-90 minutes and remains constant for at least 1 hour (Figure 20). After incubation at room temperature, the signal is measured in a luminometer.

Advantages

Galacto-*Star* chemiluminescent assay for β-galactosidase is among the most sensitive reporter gene assays available (Figure 21). As few as 10 femtograms of β-galactosidase (20,000 molecules) are detectable [9]. High sensitivity makes this system ideal for detection of weak expression and for transfection normalization with other sensitive reporter gene assays. Colorimetric, fluorometric, and isotopic assays cannot rival the dynamic range of Galacto-*Star* Assay System, which spans femtogram to nanogram levels of protein. Unlike Galacto-Light™ and Galacto-Light Plus™ systems (see page 16) which require the sequential addition of substrate and enhancer, Galacto-*Star* Assay System is a one-step assay procedure, following lysate preparation, simplifying the detection procedure. The chemiluminescent assay is compatible with lysis buffers used with luciferase assays, making it ideal for transfection normalization.

Applications

The Galacto-*Star* system is formatted with a choice of lysis buffers for use in mammalian or yeast cells. The Galacto-*Star* assay system is used widely for traditional reporter gene assays in transfected mammalian cells [3, 4, 11], and in insect cells [16]. A wide variety of applications have been performed, including viral function assays with β-gal-encoding pseudovirions [17] and MAGI cells [6, 8], normalization of siRNA transfection [7], and as a reporter read-out for epitope recognition by an engineered CTL hybridoma cell line [15]. The Galacto-*Star* system has been used to assay tissue extracts of transgenic mice made with β-gal-tagged mouse embryonic stem cells [5, 12]. The system is also formatted for use with yeast cells, and is ideally suited for reporter gene assays in yeast [10], or the study of protein:protein interactions with the yeast two-hybrid system [2, 13]. Galacton-*Star* substrate has been used for reporter gene assays in bacterial cells with modified lysis reagents [18].

Two novel applications demonstrated have been a cell death assay, by measurement of β-gal reporter enzyme released into culture media [14], and a stop codon read-through assay using a constitutively-expressed β-gal-luciferase fusion construct [1].

The Galacto-*Star* assay system has wide application to assays that use β-gal reporter as a read-out for gene expression in many cell types and tissues from whole animals, or as a functional read-out for viral function, immune cell activation, cell death, and mRNA processing.

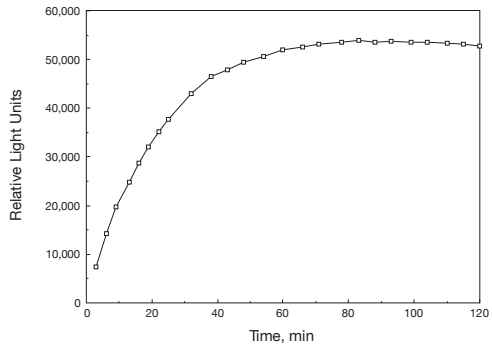

Figure 20. Detection of β-Galactosidase with Galacto-*Star*™ Assay System.

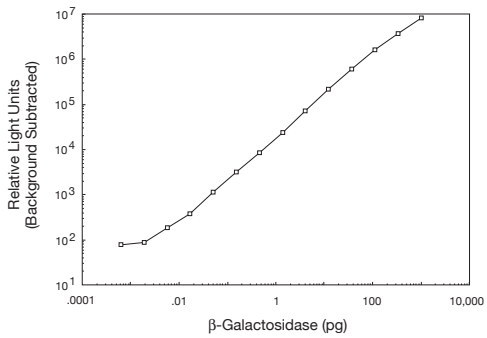

Figure 21. Sensitivity of Galacto-*Star*™ Assay System.

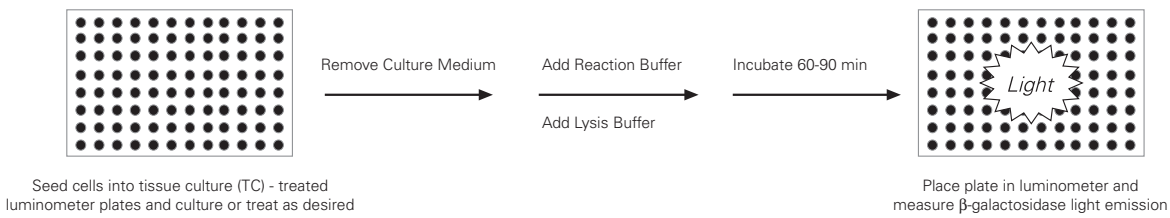

Figure 22. β-Galactosidase Reporter Gene Assay with Galacto-*Star*™ System.

Galacto-*Star*™ System Assay System, continued

One-Step β–galactosidase reporter gene assay

Product Configuration

Galacto-*Star*™ System Standard Size

T1012 – Mammalian Cells
T1019 – Yeast Cells
Capacity: 200 single tube assays
Capacity: 600 assays with microplate format
Contents:
- 1.2 mL Galacton-*Star*® substrate
- 60 mL Reaction Buffer Diluent with Sapphire-II™ enhancer
- 70 mL Mammalian Lysis Solution or 80 mL 5X Z Yeast Lysis Buffer

Galacto-*Star*™ System Large Size

T1014 – Mammalian Cells
T1021 – Yeast Cells
Capacity: 600 single tube assays
Capacity: 1,800 assays with microplate format
Contents:
- 3.6 mL Galacton-*Star*® substrate
- 180 mL Reaction Buffer Diluent containing Sapphire-II™ enhancer
- 210 mL Mammalian Lysis Solution or 240 mL 5X Z Yeast Lysis Buffer

Galacto-*Star*™ System Screening Size

T1013 – Mammalian Cells
T1020 – Yeast Cells
Capacity: 15,000 assays with microplate format
Contents:
- 30 mL Galacton-*Star*® substrate
- 1.5 L Reaction Buffer Diluent containing Sapphire-II™ enhancer
- 1.75 L Mammalian Lysis Solution or 2 L 5X Z Yeast Lysis Buffer

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Galacto-*Star*™ Mammalian Cell Reporter Gene Assay System	standard size	T1012
Galacto-*Star*™ Mammalian Cell Reporter Gene Assay System	large size	T1014
Galacto-*Star*™ Mammalian Cell Reporter Gene Assay System	screening size	T1013
Galacto-*Star*™ Yeast Cell Reporter Gene Assay System	standard size	T1019
Galacto-*Star*™ Yeast Cell Reporter Gene Assay System	large size	T1021
Galacto-*Star*™ Yeast Cell Reporter Gene Assay System	screening size	T1020
Galacto-*Star*™ Reaction Buffer Diluent with Galacton-*Star*® Chemiluminescent Substrate	180 mL 3.6 mL	T1056

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β-Galactosidase (Galacto-*Star*™ Reporter Gene Assay System; for a complete reference list, please see the Applied Biosystems website.)

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Galacto-Light™ and Galacto-Light Plus™ Assay Systems

β-Galactosidase reporter gene assay

Description

Galacto-Light™ and Galacto-Light Plus™ assay systems are designed for rapid, ultrasensitive β-galactosidase reporter gene assays. These chemiluminescent assays exhibit over three orders of magnitude greater sensitivity compared to colorimetric β-galactosidase assays and are performed in a fraction of the time required for assays for chloramphenicol acetyl transferase (CAT) and ELISA-based reporter assays.

Galacto-Light Assay Systems includes Galacton® chemiluminescent β-galactosidase substrate and requires the use of a luminometer equipped with an injector. Galacto-Light Plus Assay systems includes Galacton-Plus® substrate and may be used in tube luminometers without injectors. For use with a microplate luminometer, an automated injector is highly recommended for accurate results. Galacton-Plus substrate exhibits prolonged signal emission compared with Galacton substrate (Figure 23). The Reaction Buffer is designed to aid in discrimination of bacterial β-galactosidase reporter activity from endogenous mammalian activity. This enables sensitive detection even in cell lines with relatively high levels of endogenous β-galactosidase activity [3].

Advantages

Galacto-Light and Galacto-Light Plus Assay Systems for β-galactosidase are among the most sensitive reporter gene assays available. As few as 2 femtograms of β-galactosidase (2,600 molecules) are detectable with Galacto-Light Plus Assay System [3]. High sensitivity allows detection of weak expression. Colorimetric, fluorometric, and isotopic assays cannot rival the dynamic range of Galacto-Light and Galacto-Light Plus Assay Systems, which ranges from femtogram to nanogram levels of protein (Figure 24). Galacto-Light Plus Assay System shows up to a five-fold increase in signal-to-noise over Galacto-Light Assay System, making it more suitable for monitoring lower levels of reporter activity. The assays use a lysis solution compatible with luciferase assays, making them ideal for transfection normalization; both assays are performed rapidly in a luminometer with comparable levels of sensitivity. Both assays require addition of two reagents following lysate preparation; one reagent addition requires the use of a luminometer with an automatic injector for microplate assays. New users are recommended to use the Galacto-*Star*™ or Gal-Screen® Assay Systems, which provide simpler assay protocols without the need for injection, while providing identical high sensitivity detection.

Applications

The Galacto-Light and Galacto-Light Plus Assay Systems are widely used for traditional reporter gene assays in transfected mammalian cell lines in culture [14, 7, 19], primary culture cells [8], tissue extracts from transgenic mice [17], frog embryo extracts [10], and *Drosophila* embryo extracts [15]. A variety of applications have been performed, such as viral function assays with β-gal-encoding MAGI cells [16], and targeted gene expression for gene therapy [6]. These assay systems have also been utilized in yeast reporter gene assays, including *Schizosaccharomyces* [11] and *Candida* [23], and for the study of protein:protein interactions with the yeast two-hybrid system [1, 6] and DNA:protein interactions with the one-hybrid system [24]. Galacto-Light Plus assay system has been used for reporter gene assays in *Pseudomonas* bacterial cells [20].

Several novel applications have been performed with the Galacto-Light assay systems, including cytotoxicity, by measurement of β-gal reporter

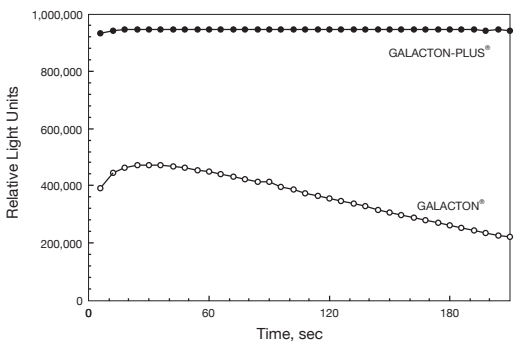

Figure 23. Detection of β-Galactosidase with Galacton® and Galacton-Plus® Substrates.

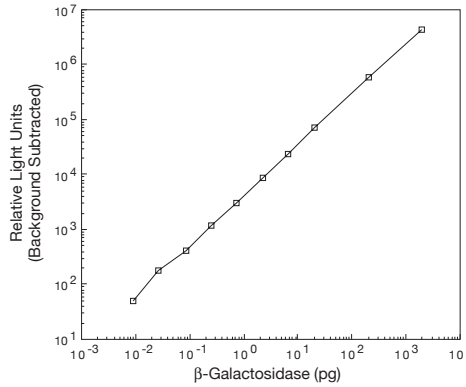

Figure 24. Detection of β-Galactosidase with Galacto-Light™ System.

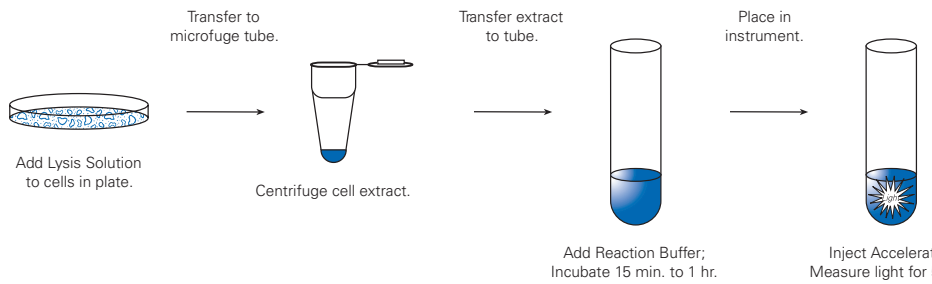

Figure 25. β-Galactosidase Reporter Gene Assay with Galacto-Light Plus™ System.

Galacto-Light™ and Galacto-Light Plus™ Assay Systems, continued

β-Galactosidase reporter gene assay

enzyme released into culture media [12], and an RNA trans-splicing assay performed in primary fetal fibroblasts by measuring reconstitution of β-gal activity from partial transcripts [13]. These assays provide highly sensitive detection for β-galactosidase complementation assays, and have been used for intracellular detection of protein-protein interactions [22] and cell fusion [21].

The Galacto-Light™ and Galacto-Light Plus™ assay systems have wide application to assays that use the β-gal reporter enzyme as a functional read-out, enabling highly sensitive detection in many different types of cells and organisms.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Galacto-Light™ Reporter Gene Assay System	standard size	T1006
Galacto-Light™ Reporter Gene Assay System	large size	T1010
Galacto-Light™ Reporter Gene Assay System	screening size	T1008
Galacto-Light Plus™ Reporter Gene Assay System	standard size	T1007
Galacto-Light Plus™ Reporter Gene Assay System	large size	T1011
Galacto-Light Plus™ Reporter Gene Assay System	screening size	T1009
Galacto-Light™ Reaction Buffer Diluent with Galacton® Chemiluminescent Substrate	120 mL 1.2 mL	T1054
Galacto-Light™ Reaction Buffer Diluent with Galacton-Plus® Chemiluminescent Substrate	120 mL 1.2 mL	T1055
Light Emission Accelerator (Galacto-Light™)	210 mL	T2084
Light Emission Accelerator-II (Galacto-Light Plus™)	210 mL	T2222

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Product Configuration

Galacto-Light™ or Galacto-Light Plus™ System Standard Size

T1006 – Galacto-Light™ System
T1007 – Galacto-Light Plus™ System
Capacity: 200 single tube assays
Capacity: 600 assays with microplate format
Contents:
- 0.4 mL Galacton® (Galacto-Light™) or Galacton-Plus® substrate (Galacto-Light Plus™)
- 40 mL Reaction Buffer Diluent
- 70 mL Light Emission Accelerator (Galacto-Light™) or Light Emission Accelerator-II (Galacto-Light Plus™)
- 70 mL Lysis Solution

Galacto-Light™ or Galacto-Light Plus™ System Large Size

T1010 – Galacto-Light™ System
T1011 – Galacto-Light Plus™ System
Capacity: 600 single tube assays
Capacity: 1800 assays with microplate format
Contents:
- 1.2 mL Galacton® (Galacto-Light™) or Galacton-Plus® substrate (Galacto-Light Plus™)
- 120 mL Reaction Buffer Diluent
- 210 mL Light Emission Accelerator (Galacto-Light™ System) or Light Emission Accelerator-II (Galacto-Light Plus™)
- 210 mL Lysis Solution

Galacto-Light™ or Galacto-Light Plus™ System Screening Size

T1008 – Galacto-Light™ System
T1009 – Galacto-Light Plus™ System
Capacity: 15,000 assays with microplate format
Contents:
- 10 mL Galacton® (Galacto-Light™) or Galacton-Plus® substrate (Galacto-Light Plus™)
- 1 L Reaction Buffer Diluent
- 1.75 L Light Emission Accelerator (Galacto-Light™) or Light Emission Accelerator-II (Galacto-Light Plus™)
- 1.75 L Lysis Solution

β-Galactosidase (Galacto-Light™ Reporter Gene Assay System; for a complete reference list, please see the Applied Biosystems website.)

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β-Galactosidase (Galacto-Light Plus™ Reporter Gene Assay System; for a complete reference list, please see the Applied Biosystems website.)

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Luc-Screen® Assay System

Extended-glow firefly luciferase reporter gene assay

Description

The Luc-Screen® assay system with extended-glow light emission is designed for sensitive detection of firefly luciferase reporter enzyme, especially for high throughput screening assays. Luciferase is an ideal reporter due to the high sensitivity of detection and the absence of endogenous luciferase activity in mammalian cells. Luc-Screen conveniently couples in-well cell lysis in the presence of culture medium with a high sensitivity assay that exhibits extended-glow light emission kinetics. Light signal can be measured between 10 minutes and several hours after adding assay reagents. Luc-Screen system is designed for maximum assay flexibility in a high-throughput format and can be used in luminometers without automatic injectors.

Luc-Screen system is formulated to provide a convenient and easy-to-use firefly luciferase assay that is optimized for use in high-throughput screening. Cells are seeded into opaque white tissue culture (TC)-treated microplates (not supplied) or clear-bottom/opaque white side TC-treated microplates, if desired. The system has two reagents that are added to cells in culture medium (with or without phenol red) in microplate wells. Although the presence of phenol red causes some decrease in signal intensity, assay sensitivity remains unaffected. Cell lysis occurs during an initial 10 minute incubation; within this time, light signal reaches plateau. Light emission persists with a signal half-life of 4-5 hours, providing flexibility in the time between reagent addition and measurement.

Advantages

Luciferase is one of the most sensitive reporter enzymes available. The Luc-Screen® system detects fewer than 50 femtograms of pure enzyme in culture medium samples (Figure 26). The high sensitivity of Luc-Screen® is complemented by a wide dynamic range. A linear signal is obtained with the Luc-Screen assay from 50 femtograms to 100 nanograms of pure enzyme in culture medium, a dynamic range of six orders of magnitude.

Applications

The Luc-Screen reporter gene assay system is ideal for high throughput firefly luciferase reporter gene expression assays in mammalian cells, and has been used for gene expression assays [1], compound screening [3]and large-scale promoter function assays [2].

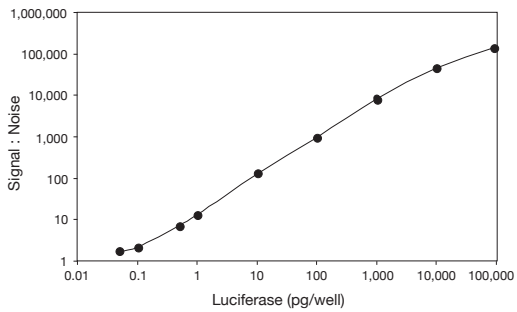

Figure 26. Sensitivity of Luc-Screen® Assay. Serial dilutions of purified luciferase, prepared in DMEM, were used for Luc-Screen assays. Signals were measured on a microplate luminometer.

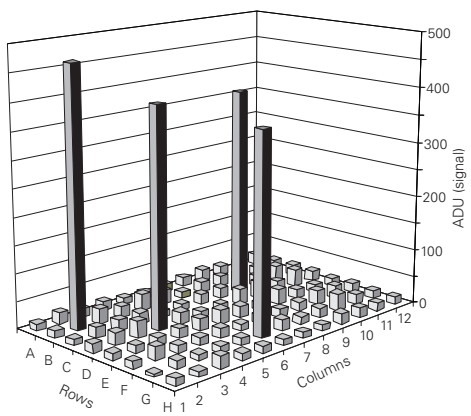

Figure 27. Luc-Screen® Assay: Forskolin Induction of pCRE-Luc Transfected Cells. Cells were transfected with a luciferase construct under the control of a cAMP-responsive promoter, and were then seeded into a 96-well plate. Several wells were treated with forskolin to induce cAMP and stimulate luciferase induction in a screening-like assay. Measurements were performed on a CCD microplate luminometer.

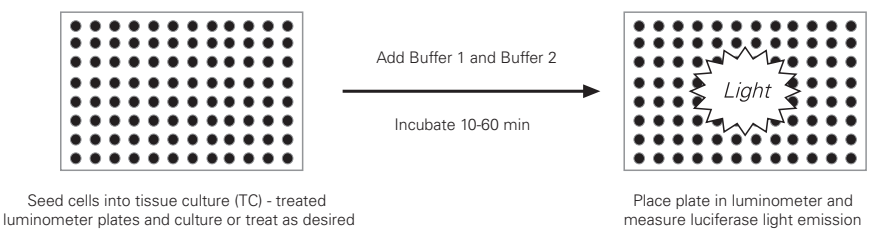

Figure 28. Luc-Screen® Reporter Gene Assay.

Luc-Screen® Assay System, continued

Extended-glow firefly luciferase reporter gene assay

Product Configuration

Luc-Screen® reagents come as two buffers which are added to culture medium in a 2:1:1 (culture medium : Buffer 1 : Buffer 2) ratio. Luc-Screen system is available in several sizes so that customers can match their needs with an appropriate kit.

	ASSAYS PER KIT	BUFFER 1	BUFFER 2
T1035	200	2 x 5 mL	2 x 5 mL
T1033	1,000	2 x 25 mL	2 x 25 mL
T1036	5,000	1 x 250 mL	1 x 250 mL
T1034	10,000	2 x 250 mL	2 x 250 mL

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Luc-Screen® Extended-Glow Firefly Luciferase Assay System	200 assays	T1035
Luc-Screen® Extended-Glow Firefly Luciferase Assay System	1,000 assays	T1033
Luc-Screen® Extended-Glow Firefly Luciferase Assay System	5,000 assays	T1036
Luc-Screen® Extended-Glow Firefly Luciferase Assay System	10,000 assays	T1034

For Research Use Only. Not for use in diagnostic procedures.

Luciferase (Luc-Screen® Reporter Gene Assay System;
(For a complete reference list, please see the Applied Biosystems website.)

1. Ahmed CM, Wills KN, Sugarman BJ, Johnson DE, Ramachandra M, Nagabhushan TL, Howe JA (2001) Selective expression of nonsecreted interferon by an adenoviral vector confers antiproliferative and antiviral properties and causes reduction of tumor growth in nude mice, *Journal of Interferon and Cytokine Research* **21:**399-408.
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Luciferase Assay System

Luciferase reporter gene assay system

Description

Luciferase Assay System is a bioluminescent assay system designed for rapid, sensitive detection of firefly luciferase expressed by transfected cells. The kit incorporates the substrate luciferin and proprietary reagents to enhance light emission. This enhanced luciferase/luciferin reaction produces a light signal that decays with a half-life of approximately 5 minutes. The assay is compatible with the lysis buffer included in Tropix β-galactosidase assay kits, making this assay ideal for co-transfections.

Cell lysate is mixed with Substrate A, which contains reagents necessary for the luciferase reaction. Light signal from the luciferase enzyme present in the extract is measured immediately after the injection of Substrate B, containing luciferin.

Advantages

The high sensitivity and absence of endogenous luciferase activity in the majority of cell types makes luciferase an excellent reporter enzyme. The wide dynamic range of the assay enables accurate measurement of luciferase concentration from the femtogram to nanogram range (Figure 29). The wide dynamic range of seven orders of magnitude cannot be rivaled by fluorimetric or colorimetric assays for other reporter proteins.

Applications

The Luciferase Assay System provides a traditional flash kinetics luciferase assay system for measuring gene expression from a firefly luciferase reporter construct in mammalian cells [1, 2]. This assay system requires the use of a luminometer with injection capabilities, and the reaction kinetics provide a very rapid assay read-out.

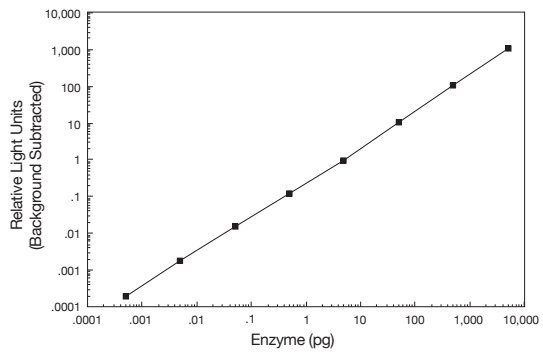

Figure 29. Detection Limit and Dynamic Range of Luciferase Assay Kit.

Product Configuration

Luciferase Assay System Standard Size

T1000
Capacity: 200 assays
Contents:
- 20 mL of Luciferase Assay System Substrate A*
- 20 mL of Luciferase Assay System Substrate B*
- 70 mL of Luciferase Assay System Lysis Solution

Luciferase Assay System Large Size

T1002
Capacity: 600 assays
Contents:
- 3 x 20 mL of Luciferase Assay System Substrate A*
- 3 x 20 mL of Luciferase Assay System Substrate B*
- 210 mL of Luciferase Assay System Lysis Solution

Luciferase Assay System Screening Size

T1001
Capacity: 5,000 assays
Contents:
- 5 x 100 mL of Luciferase Assay System Substrate A*
- 5 x 100 mL of Luciferase Assay System Substrate B*
- 1.75 L of Luciferase Assay System Lysis Solution

*Supplied lyophilized

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Luciferase Reporter Gene Assay System	standard size	T1000
Luciferase Reporter Gene Assay System	large size	T1002
Luciferase Reporter Gene Assay System	screening size	T1001

For Research Use Only. Not for use in diagnostic procedures.

Luciferase (Luciferase Assay System; for a complete reference list, please see the Applied Biosystems website.)

1. Poser S, Impey S, Xia Z, Storm DR (2003) Brain-derived neurotrophic factor protection of cortical neurons from serum withdrawal-induced apoptosis is inhibited by cAMP *J Neuroscience* **23(11):**4420-4427.
2. Singh RP, Dhawan P, Golden C, Kapoor GS, Mehta KD (1999) One-way cross-talk between p38MAPK and p42/44MAPK, *J Biol Chem* **274(28):**19593-19600

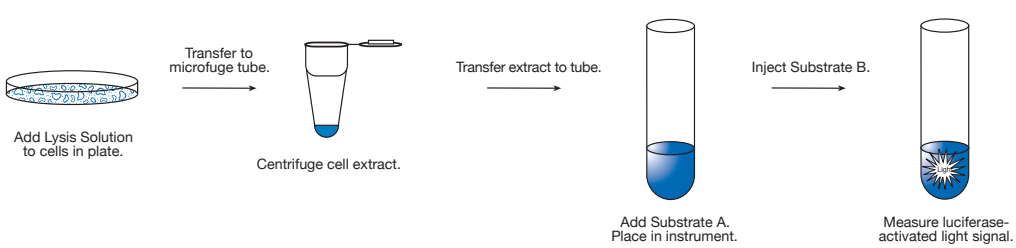

Figure 30. Luciferase Reporter Gene Assay.

Dual-Light® Assay System

Combined firefly luciferase and β-Galactosidase reporter gene assay

Description

Dual-Light® luminescent reporter gene assay is designed for the rapid and sensitive detection of firefly luciferase and β-galactosidase in the same sample. The use of two reporter genes, one as an experimental reporter and the other as a constitutively-expressed transfection control reporter, is very widely used and is often necessary to accurately quantitate activity from experimental reporter constructs. This assay enables the measurement of firefly luciferase and β-galactosidase in a single aliquot of cell lysate. Luciferase is typically used as the experimental reporter, and β-galactosidase is typically quantitated from a co-transfected constitutive expression vector to determine transfection efficiency. The luciferase measurement is then normalized to the β-galactosidase measurement. Light signal from each enzymatic reaction is measured sequentially in a luminometer with automatic injectors.

First, luciferase reporter enzyme activity is quantitated with an enhanced luciferase reaction. Following a 30-60 minute incubation and addition of a light emission accelerator, β-galactosidase reporter enzyme activity is determined with Galacton-Plus® substrate.

Advantages

Both reporter enzyme measurements are combined into a single sequential assay protocol using only one aliquot of extract for greater convenience and precision. The entire assay is completed in less than one hour. The wide dynamic range (Figure 31) of this dual assay enables accurate measurement of firefly luciferase and β-galactosidase concentrations over seven orders of magnitude, from the femtogram to nanogram range [9].

Applications

Dual-Light® reporter gene assay system has been very widely used for reporter quantitation/transfection normalization from transiently transfected mammalian cell lines [1, 5, 6, 7, 8], as well as transfected primary cells [2, 3, 4, 10}. In addition, it has been used with a modified lysis buffer to quantitate luciferase and β-galactosidase activities from a novel reporter fusion construct in yeast cells [11].

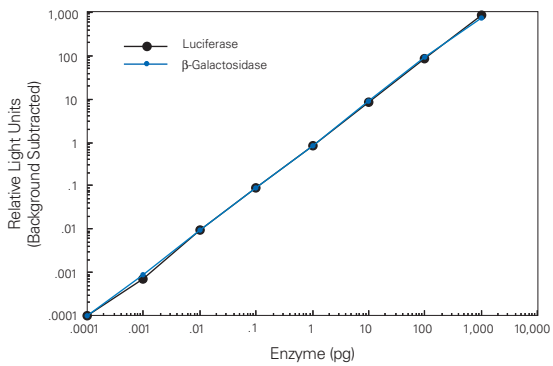

Figure 31. Detection of Firefly Luciferase and β-Galactosidase with Dual-Light® System.

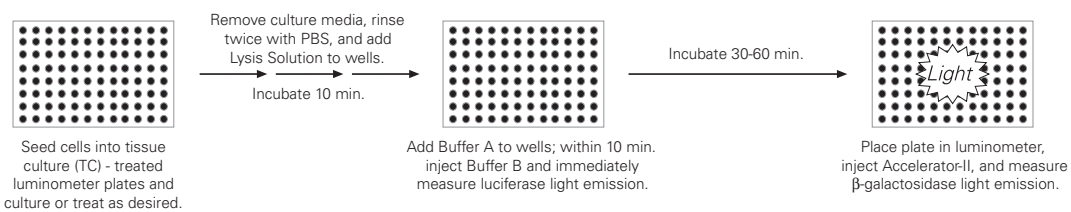

Figure 32. Firefly Luciferase and β-Galactosidase Reporter Gene Assays with Dual-Light® System.

Dual-Light® Assay System, continued

Combined firefly luciferase and β-Galactosidase reporter gene assay

Product Configuration

Dual-Light® System Standard Size

T1003
Capacity: 200 combined assays
Contents:
- 0.2 mL Galacton-Plus® substrate
- 5 mL Buffer A*
- 22 mL Buffer B*
- 25 mL Light Emission Accelerator-II
- 70 mL Lysis Solution

Dual-Light® System Large Size

T1005
Capacity: 600 combined assays
Contents:
- 0.6 mL Galacton-Plus® substrate
- 3 x 5 mL Buffer A*
- 3 x 22 mL Buffer B*
- 75 mL Light Emission Accelerator-II
- 210 mL Lysis Solution

Dual-Light® System Screening Size

T1004
Capacity: 4,000 combined assays
Contents:
- 4 mL Galacton-Plus® substrate
- 20 x 5 mL Buffer A*
- 20 x 22 mL Buffer B*
- 500 mL Light Emission Accelerator-II
- 1.4 L Lysis Solution

*Supplied lyophilized

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Dual-Light® Reporter Gene Assay System	standard size	T1003
Dual-Light® Reporter Gene Assay System	large size	T1005
Dual-Light® Reporter Gene Assay System	screening size	T1004
Dual-Light® Buffer A	5 mL	T2176
Dual-Light® Buffer B with Galacton-Plus® Substrate	22 mL/0.2 mL	T1053
Light Emission Accelerator-II	210 mL	T2222

For Research Use Only. Not for use in diagnostic procedures.

β-Galactosidase/Luciferase (Dual-Light® Reporter Gene Assay System; for a complete reference list, please see the Applied Biosystems website.)

1. Babb R, Bowen BR (2003) SDP1 is a peroxisome-proliferator-activated receptor g2 co-activator that binds through its SCAN domain, *Biochem J* **370:**719-727.
2. Bourcier T, Sukhova G, Libby P (1997) The nuclear factor k-B signaling pathway participates in dysregulation of vascular smooth muscle cells in vitro and in human atherosclerosis, *J Biol Chem* **272(25):**15817-15824.
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9. Martin CS, Wight PA, Dobretsova A, Bronstein I.(1996) Dual luminescence-based reporter gene assay for luciferase and b-galactosidase, *BioTechniques* **21(3):**520-524.
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11. Williams I, Richardson J, Starkey A, Stansfield I (2004) Genome-wide prediction of stop codon readthrough during translation in the yeast Saccharomyces cerevisiae, *Nucl Acids Res* **32(22):**6605-6616.

Phospha-Light™ Assay System

Secreted placental alkaline phosphatase (SEAP) reporter gene assay

Description

Phospha-Light™ assay system is a chemiluminescent reporter gene assay for the sensitive detection of secreted placental alkaline phosphatase (SEAP). SEAP is a reporter protein that is secreted into the cell culture media and detected by testing aliquots of media [2]. The SEAP gene product is a truncated form of human placental alkaline phosphatase. Detection of non-secreted placental alkaline phosphatase (PLAP) is also possible [16].

A combination of proprietary Tropix® reagents provides highly sensitive detection with the Phospha-Light Assay System. The system incorporates CSPD® high performance alkaline phosphatase substrate, Emerald™ luminescence enhancer, and a unique buffer system designed to specifically inhibit endogenous non-placental alkaline phosphatase activity.

A sample of cell culture media is transferred to a microfuge tube and diluted with Phospha-Light dilution buffer. After heating 30 minutes at 65°C, the sample is transferred to a luminometer tube or microplate. Phospha-Light assay buffer containing differential alkaline phosphatase inhibitors (to achieve high sensitivity in media containing non-placental alkaline phosphatase) and reaction buffer containing CSPD substrate and Emerald enhancer are then added. After a 20-minute incubation at room temperature, the chemiluminescent signal is measured in a luminometer. The unique properties of human placental alkaline phosphatase, including its heat stability and resistance to L-homoarginine, enable it to be distinguished from endogenous non-placental alkaline phosphatase activity.

Light emission kinetics observed with Phospha-Light Assay System reagents are shown (Figure 34). The persistent glow of the chemiluminescent signal permits the use of simple luminometers without injectors or scintillation counters for measurement.

Advantages

Secreted reporter proteins eliminate the need for cell lysis. A population of cells can be monitored over time and remains intact for further experimentation. The Phospha-Light assay is one of the easiest and fastest methods for optimizing transfection efficiency. This chemiluminescent method allows detection of fewer than 10 femtograms of SEAP (Figure 33), which is three orders of magnitude more sensitive than colorimetric detection of SEAP. The wide linear range of six orders of magnitude of the Phospha-Light assay enables accurate intra-assay comparisons without measuring several sample dilutions.

Applications

The Phospha-Light reporter gene assay system has been used widely for reporter gene assays to measure gene expression in established cell lines [4] and in transfected primary cells [15, 21], including as a gene knockdown/RNA interference read-out [5]. The Phospha-Light reporter gene assay has been used for a wide variety of viral functional assays, including viral gene expression assays [8], viral replication [9], viral fusogenicity [1], virus neutralization and viral-mediated cell-cell fusion [10], and viral infectivity [14]. Use of the SEAP reporter protein is very enabling for in vivo reporter gene assays, by assaying serum samples from transgenic, transfected or viral vector-infected animals. The Phospha-Light reporter gene assay system has been used to measure SEAP levels in sera from transgenic or transfected whole animals, including mouse [18], rat [17], marmoset [7], monkey [13] and pig sera [12], and in chicken egg allantoic fluid [22]. The mouse SEAP protein (mSEAP) has recently been developed for improved SEAP protein stability in transgenic mice, and the Phospha-Light™ system has been used for sensitive detection of mSEAP [18].

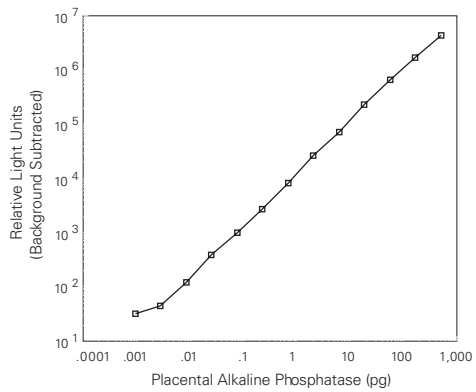

Figure 33. Detection of Placental Alkaline Phosphatase with Phospha-Light™ System.

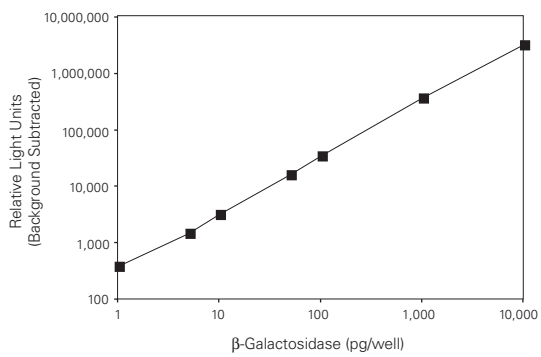

Figure 34. Phospha-Light™ System Light Emission Kinetics.

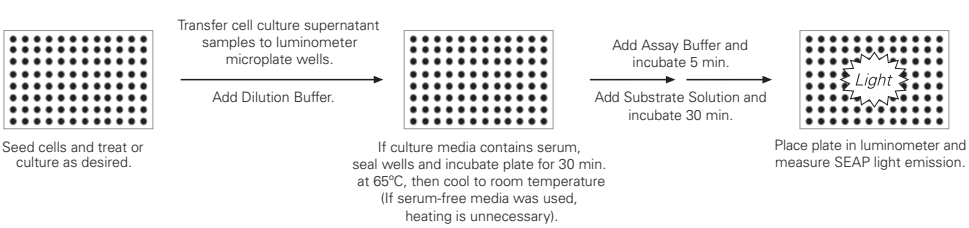

Figure 35. Secreted Alkaline Phosphatase Reporter Gene Assay with Phospha-Light™ System.

Phospha-Light™ Assay System, continued

Secreted placental alkaline phosphatase (SEAP) reporter gene assay

In addition to reporter gene (gene expression) applications, the Phospha-Light assay system is used to measure SEAP as a functional reporter for receptor-ligand binding assays with a SEAP-ligand chimera [20], protease-mediated secretion [19], and for secretion pathway activity [11]. Finally, the Phospha-Light™ assay system has also been used for the cellular measurement of non-placental alkaline phosphatase as a biomarker [4].

Sensitive detection of SEAP or PLAP reporter protein with the Phospha-Light assay system enables a large number of applications in many areas of life science research, including gene expression, viral function assays, vaccine development, development of viral vectors and gene delivery methods for gene therapy, in vivo gene expression monitoring and novel cellular functional assays.

Product Configuration

Phospha-Light™ System Standard Size

T1015

Capacity: 200 single tube assays (triplicate assays of 66 samples or duplicate assays of 83 samples)
Capacity: 400 assays with microplate format (triplicate assays of 133 samples or duplicate assays of 166 samples)
Contents:

- 1.0 mL CSPD® chemiluminescent substrate
- 19 mL Phospha-Light™ Reaction Buffer Diluent with Emerald™ enhancer
- 20 mL Phospha-Light™ Assay Buffer
- 5 mL 5X Dilution Buffer
- 50 µL Positive Control Placental Alkaline Phosphatase

Phospha-Light™ System Large Size

T1017

Capacity: 600 single tube assays (triplicate assays of 200 samples or duplicate assays of 250 samples)
Capacity: 1,200 assays with microplate format (triplicate assays of 400 samples or duplicate assays of 500 samples)
Contents:

- 3.0 mL CSPD® chemiluminescent substrate
- 57 mL Phospha-Light™ Reaction Buffer Diluent with Emerald™ enhancer
- 60 mL Phospha-Light™ Assay Buffer
- 15 mL 5X Dilution Buffer
- 50 µL Positive Control Placental Alkaline Phosphatase

Phospha-Light™ System Screening Size

T1016

Capacity: 10,000 assays with microplate format (triplicate assays of 3,333 samples or duplicate assays of 4,166 samples)
Contents:

- 25 mL CSPD® chemiluminescent substrate
- 475 mL Phospha-Light™ Reaction Buffer Diluent with Emerald™ enhancer
- 500 mL Phospha-Light™ Assay Buffer
- 125 mL 5X Dilution Buffer
- 425 µL Positive Control Placental Alkaline Phosphatase

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Phospha-Light™ Reporter Gene Assay System	standard size	T1015
Phospha-Light™ Reporter Gene Assay System	large size	T1017
Phospha-Light™ Reporter Gene Assay System	screening size	T1016

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Secreted Placental Alkaline Phosphatase (Phospha-Light™ Reporter Gene Assay System; for a complete reference list, please see the Applied Biosystems website.)

1. Alexander L, Illyinskii PO, Lang SM, Means RE, Lifson J, Mansfield K, Desrosiers RC (2003) Determinanants of increased replicative capacity of serially passaged Simian Immunodeficiency Virus with nef deleted in Rhesus monkeys, *J Virol* **77(12):**6823-6835.
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3
Immunodetection Products

Introduction to Chemiluminescent ELISAs

ELISA

Enzyme-linked immunosorbent assays (ELISAs) are used for sensitive analyte detection. ELISAs can be formatted in several configurations on a variety of solid supports.

A direct sandwich ELISA is often used for the detection of larger molecules with multiple antigenic sites, usually a protein. In this format (Figure 36, Panel A), a solid support is coated with a capture antibody specific for the protein of interest; the capture antibody forms an immunocomplex with the antigen from the sample; a detector antibody covalently derivatized with alkaline phosphatase, specific for a second antigenic site on the captured protein, is then added. After washing, substrate with enhancer is added. The enzyme-generated signal is proportional to the concentration of antigen.

A variation of this assay is used to screen hybridomas for the production of monoclonal antibodies (Figure 36, Panel B). In this case, the solid phase is coated with antigen, hybridoma culture supernatant containing a monoclonal antibody is added, and then an enzyme-labeled secondary antibody specific for the monoclonal antibody is used to detect the antigen-captured monoclonal antibody.

Competitive immunoassays can be set up in two modes (Figure 36, Panel C and D). In these assays, a competition occurs between bound and free antigen, or labeled and unlabeled antigen, for available antibody binding sites. This type of assay results in an inverse standard curve. A decrease in signal is observed for increasing concentrations of antigen.

Chemiluminescent Immunoassays

The use of 1,2-dioxetane enzyme substrates permits the ultrasensitive detection of analytes by ELISA. The most common enzyme used in conjunction with 1,2-dioxetane substrates for ELISA applications is alkaline phosphatase. Tropix® 1,2-dioxetane substrates have been used for detecting thyroid-stimulating hormone (TSH) with a commercially available ELISA employing an alkaline phosphatase label [2]. The results obtained with CSPD® substrate with Sapphire-II™ enhancer (Figure 37) show a significant improvement in signal-to-background performance at all concentrations of TSH compared to those obtained with the colorimetric substrate, *p*-nitrophenyl phosphate (pNPP). This benefit can be expected when a colorimetric direct sandwich ELISA is converted to 1,2-dioxetane/enhancer chemiluminescence. Sandwich immunoassay formats with 1,2-dioxetane substrates have been used for calculation of antigen-antibody binding constants [10], and quantitation of animal and human proteins from plasma and tissue extracts [9,15,18].

Competitive ELISAs can also be highly sensitive with the incorporation of 1,2-dioxetane substrates [1, 7, 13]. Because the standard curve in a competitive ELISA exhibits maximum signal intensity at the lowest concentrations of analyte, it may be necessary to adjust reagent concentrations to optimize detection of low analyte concentrations. The sensitivity of chemiluminescent detection permits the use of lower concentrations of capture antibody and competing antigen.

CDP-*Star*® substrate with Sapphire-II enhancer or Emerald-II™ enhancer has become widely used for immunoassay protein detection applications such as detection of viral antigens [14] and plasma proteins [9].

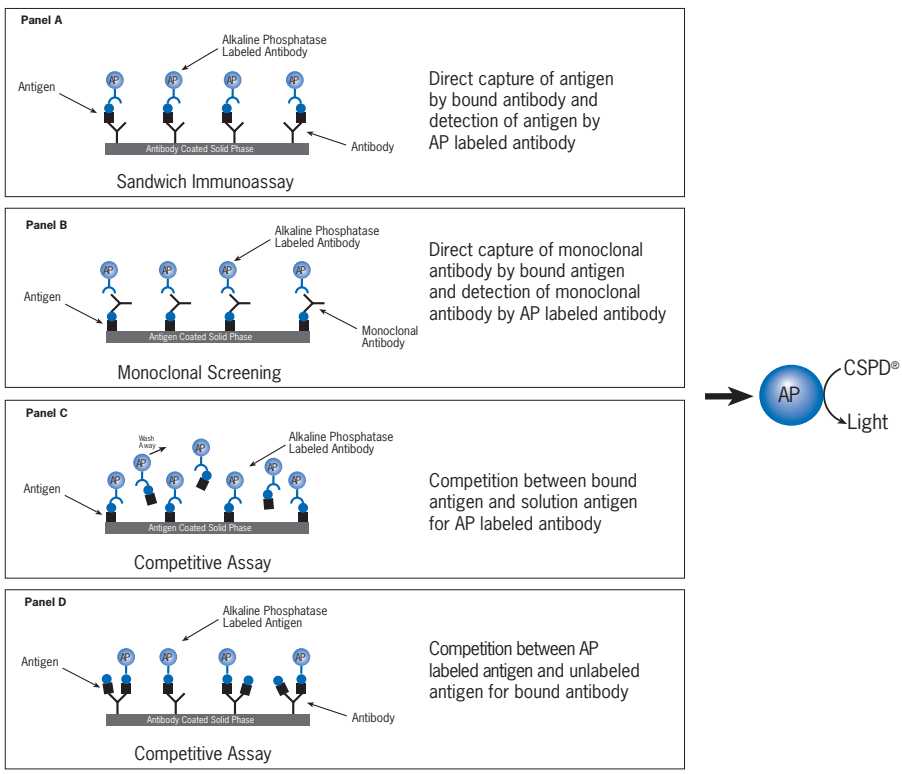

Figure 36. Immunoassay Schemes Employing an Alkaline Phosphatase Label for Detection with CSPD® Substrate.

Introduction, continued

Additional Applications

Whole-cell ELISA

Immunoassay detection of surface antigens on whole cells has been demonstrated with 1,2-dioxetane chemiluminescent detection [19]. β-galactosidase enzyme conjugates can also be used with Galacton-*Star*® substrate with Sapphire-II™ enhancer for chemiluminescent immunoassay detection, particularly for whole cell ELISA applications [12] that may exhibit high levels of cellular alkaline phosphatase.

Protein Detection Applications

Anti-phosphopeptide immunoassays with CSPD® or CDP-*Star*® substrates and Sapphire-II or Emerald-II™ enhancers have been developed for quantitation of several protein kinase activities. These include PKA, PKC, CAM-KII, receptor interacting protein and src kinases [16], WaaP protein tyrosine kinase and sugar kinase [22] and p38 kinase [8]. In addition a receptor binding assay of a neurotrophic factor to a tyrosine kinase receptor has been demonstrated [20].

Nucleic Acid and Nucleic Acid-Protein Interaction Detection Applications

DNA probe hybridization assays, DNA:protein interaction assays, and DNA aptamer binding assays are often formatted in microplate wells or on other solid phases. AP-labeled probes, hapten-labeled probes or antibodies to DNA:RNA duplexes [3] can be used to detect hybridization or binding with AP-conjugated detection reagents and 1,2-dioxetane substrates. Chemiluminescent enzyme-linked oligonucleotide assay (ELONA) has been used to quantitate DNA aptamer binding to protein [6]. CDP-*Star* substrate is used in microplate-based assay systems for detection of viral RNA or DNA by immunodetection [17, 21]. In addition, detection of chemical:DNA adducts in mammalian tissue extracts has been demonstrated using CDP-*Star* substrate with Emerald-II enhancer [5].

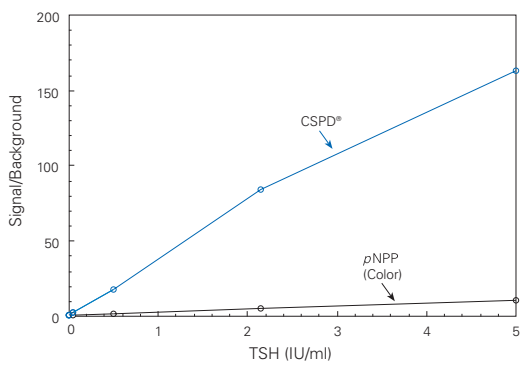

Figure 37. Comparison of Chemiluminescent and Colorimetric Detection of TSH.

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cAMP-Screen® System

Cyclic AMP immunoassay

Description

cAMP-Screen® Immunoassay System enables ultrasensitive determination of cyclic AMP (cAMP) levels in cell lysates. This competitive immunoassay is formatted with maximum flexibility to permit either manual assay or automated high-throughput screening. cAMP-Screen assay utilizes the highly sensitive chemiluminescent alkaline phosphatase (AP) substrate CSPD® with Sapphire-II™ luminescence enhancer. The Ready-to-Use substrate/enhancer reagent generates sustained-glow light emission that is measured 30 minutes after addition.

cAMP-Screen immunoassay is formulated to be compatible with automated high-throughput screening instrumentation (Figure 38). Cells are seeded into plates, cultured, and treated with test compounds as desired. Cell lysates are prepared in either the presence or absence of culture media. Lysates are incubated with a cAMP-AP conjugate and an anti-cAMP antibody in a coated microplate; the resulting immune complexes are captured in the plate. In samples without cAMP, all of the cAMP-AP conjugate is captured on the coated surface, resulting in a high signal. In the presence of cAMP, the amount of cAMP-AP conjugate captured decreases as a result of competition for binding with unlabeled cAMP, causing a reduced signal (see Figure 40); signal reduction is proportional to the amount of cAMP present in the cell lysate. After washing to remove unbound cAMP-AP, the chemiluminescent substrate is added, and the resulting glow signal is measured in a luminometer.

Advantages

Chemiluminescent detection with the cAMP-Screen assay provides the highest sensitivity of any commercially available cAMP assay. As few as 60 femtomoles of cAMP can be detected with cAMP-Screen 96-well system. cAMP-Screen system is available in both 96-well and 384-well formats. Both formats have a wide assay dynamic range with the 96-well version able to detect cAMP concentrations from 0.06 to 6,000 picomoles and the 384-well version able to detect cAMP concentrations from 0.2 to 200 picomoles (without the need for sample dilution or manipulations such as acetylation). This is especially helpful in cell-based assays, when measuring G_S- or G_I-coupled agonist stimulation and/or inhibition.

Assay precision is very high for cAMP-Screen immunoassay. Intra-assay precision for duplicate samples is typically 3% or less. To ensure optimal plate %CVs, it is imperative that the substrate/enhancer be allowed to reach the maximum glow signal. Once the substrate/enhancer reaches the glow signal, the plate can be read for hours with little or no degradation of the signal. This is useful in screening where several plates are compared to each other. In addition, the assay exhibits exceptionally low cross-reactivity with other adenosine-containing or cyclic nucleotides.

Applications

The cAMP-Screen assay system is designed for quantitation of cellular cAMP for functional assays of receptor activation. cAMP-Screen has been used with established cell lines for functional measurements with endogenous receptors, cell lines with exogenously expressed ligand receptors on the cell surface, primary cell cultures, and tissues [1] in response to treatment with the appropriate ligands. The cAMP-Screen assay system has been used for receptor characterization [5, 7], orphan receptor ligand identification [4], and the characterization of novel chimeric receptors [3]. In addition, cAMP-Screen assay system can be used for high throughput screening assays [2, 8] for compounds which stimulate or interfere with these signal transduction pathways.

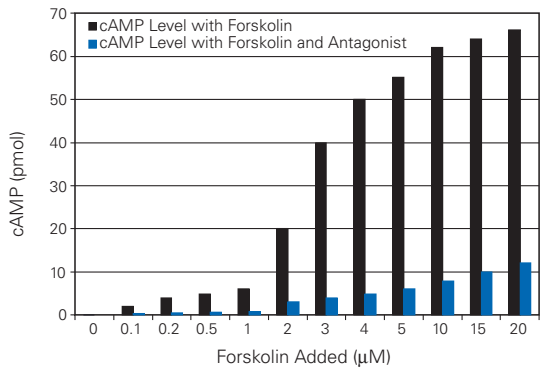

Figure 38. cAMP-Screen® Assay: Effect of Forskolin and an Antagonist on cAMP Levels. cAMP-Screen assays were performed using the indicated concentration of forskolin to induce cAMP in the absence or presence of a forskolin antagonist. Measurements were performed on a microplate luminometer.

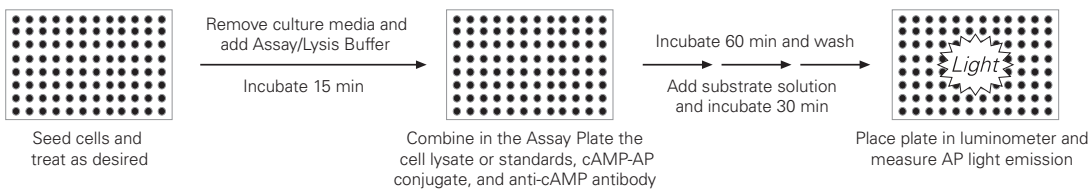

Figure 39. cAMP Immunoassay with cAMP-Screen® System.

cAMP-Screen® System, continued

Cyclic AMP immunoassay

Product Configurations

cAMP-Screen® Immunoassay System is available in either a 96-well or 384-well plate format. Each kit format is available in several sizes to provide the ideal-size kit for evaluation, R&D, or screening.

Component	T1500	T1501	T1502	T1504
Plate Format	96-well	384-well	96-well	384-well
Assays per Kit	192	768	960	19,200
Assay/Lysis Buffer	25 mL	65 mL	2 x 65 mL	2 L
cAMP Standard	2 mL	5 mL	2 x 5 mL	100 mL
Anti-cAMP Antibody	14 mL	20 mL	2 x 35 mL	500 mL
cAMP-AP Conjugate	100 mL	250 mL	2 x 250 mL	5 mL
Conjugate Dilution Buffer	10 mL	25 mL	2 x 25 mL	500 mL
Wash Buffer	500 mL (1X)	1 L (1X)	2 x 1 L (1X)	5 x 1 L (5X)
CSPD®/Sapphire-II™ RTU	25 mL	25 mL	2 x 65 mL	650 mL Substrate/Enhancer Solution
Precoated Plates	2 plates	2 plates	10 plates	50 plates

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
cAMP-Screen® 96-Well Immunoassay System	**192 assays**	**T1500**
cAMP-Screen® 384-Well Immunoassay System	**960 assays**	**T1501**
cAMP-Screen® 96-Well Immunoassay System	**768 assays**	**T1502**
cAMP-Screen® 384-Well Immunoassay System	**19,200 assays**	**T1504**
Assay/Lysis Buffer	**65 mL**	**T2327**
1X Wash Buffer	**1 L**	**T2337**
5X Wash Buffer	**1 L**	**T2356**

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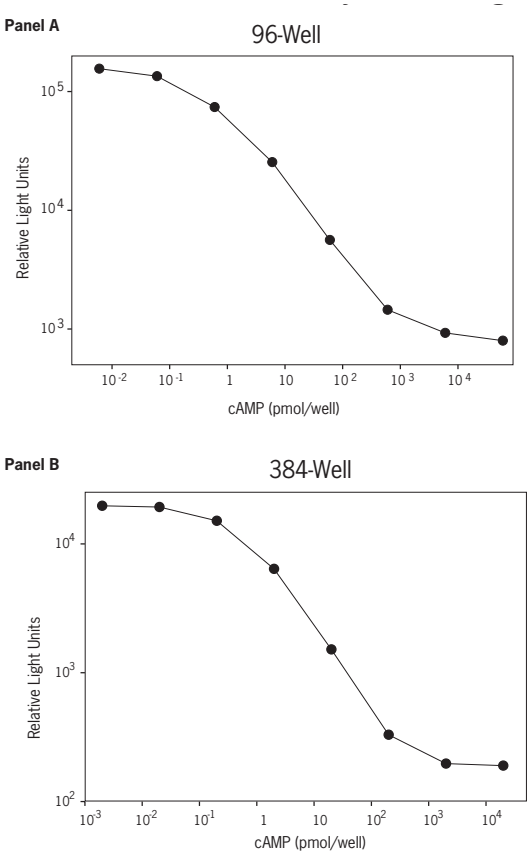

Figure 40. Dynamic Range of cAMP-Screen® Assay.

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(For a complete cAMP-Screen® Immunoassay System reference list, please see the Applied Biosystems website.)

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cAMP-Screen Direct® System

Cyclic AMP immunoassay

Description

cAMP-Screen Direct® Immunoassay System enables ultrasensitive quantitation of cyclic AMP (cAMP) levels in cell lysates. With the cAMP-Screen Direct assay system, cells are plated directly in the antibody pre-coated cAMP-Screen Direct assay plates. The pre-coated plates supplied are clear-bottom, enabling examination of cells prior to start of cAMP quantitation assay. This assay system eliminates the need for a separate cell culture plate. This competitive immunoassay is formatted with maximum flexibility to permit either manual assay or automated high-throughput screening. cAMP-Screen Direct assay system utilizes the highly sensitive chemiluminescent alkaline phosphatase (AP) substrate CSPD® with Sapphire-II™ luminescence enhancer. The Ready-to-Use substrate/enhancer reagent generates sustained-glow light emission that is measured 30 minutes after addition.

cAMP-Screen Direct immunoassay system is formatted to be compatible with automated high-throughput screening instrumentation (Figure 41). Cells are seeded into pre-coated assay plates (clear-bottom, opaque white wells), cultured, and treated with test compounds as desired. Cell lysates are then prepared in either the presence or absence of culture media, and additional cAMP-Screen Direct system reagents are added directly to the lysate in the culture/assay plate. Lysates are incubated with cAMP-AP conjugate and anti-cAMP antibody, and the resulting immune complexes are captured on the antibody-coated surface of the plate. In samples without cAMP, all of the cAMP-AP conjugate is captured on the coated surface, resulting in a high signal. In the presence of cAMP, the amount of cAMP-AP conjugate captured decreases, as a result of competition for binding with unlabeled cAMP causing a reduced signal (Figure 41); signal is inversely proportional to the amount of cAMP present in the cell lysate. After washing to remove unbound cAMP-AP, the chemiluminescent substrate/enhancer is added, and the resulting signal is measured in a luminometer without reagent injection.

Advantages

The cAMP-Screen Direct Immunoassay System offers several advantages for high throughput screening applications compared to the original cAMP-Screen® assay system. With cAMP-Screen Direct system, cells are grown directly in the cAMP quantitation assay plate without subsequent lysate transfer, eliminating the need for a separate culture plate, thereby reducing total assay costs, simplifying automation requirements for assay execution, and offering increased intra-assay accuracy. Chemiluminescent detection employed by cAMP-Screen Direct assay provides identical sensitivity to the cAMP-Screen system, the highest sensitivity of any commercially available cAMP assay. As with the cAMP-Screen system, as few as 60 femtomoles of cAMP can be detected with cAMP-Screen Direct 96-well system. The cAMP-Screen Direct system is available in both 96-well and 384-well formats.

Both the cAMP-Screen and cAMP-Screen Direct assay formats have a wide assay dynamic range with the 96-well version able to detect cAMP concentrations from 0.06 to 6,000 picomoles and the 384-well version able to detect cAMP concentrations from 0.2 to 200 picomoles (without the need for sample dilution or manipulations such as acetylation).

Assay precision is very high for cAMP-Screen Direct Immunoassay System. Intra-assay precision for duplicate samples is typically 3% or less. In addition, the assay exhibits exceptionally low cross-reactivity with other adenosine-containing or cyclic nucleotides.

For cells requiring specialized growth surfaces, we do not recommend use of cAMP-Screen Direct microplates, or making surface modifications of the cAMP-Screen Direct microplates. In this case, the appropriate surface-modified plates should be used for cell growth, followed by lysate preparation and use of cAMP-Screen assay system.

Applications

The cAMP-Screen Direct assay system is designed for quantitation of cellular cAMP for functional assays of receptor activation. cAMP-Screen Direct system is designed for use with established cell lines for functional measurements with endogenous receptors, cell lines with exogenously expressed ligand receptors, and primary cell cultures [1] in response to treatment of cells with the appropriate ligands. cAMP-Screen Direct system is particularly suited for high throughput screening applications for identification of compounds which stimulate or interfere with receptor function and signal transduction.

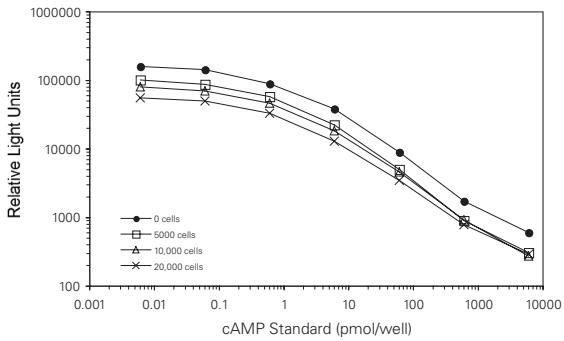

Figure 41. Sensitivity of the cAMP-Screen Direct® System. cAMP assay performed with the cAMP-Screen Direct system. cAMP standards were used in a cAMP-Screen Direct assay plate following growth of different densities of HEK293 cells in assay wells. Detection sensitivity of exogenously added cAMP is unchanged following growth of cells in assay plate. Signal intensity differences result from basal cellular levels of cAMP. Signal was measured with the TR717™ microplate luminometer.

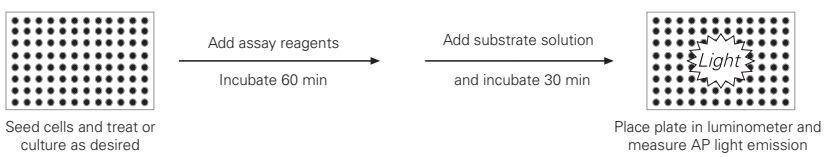

Figure 42. cAMP Immunoassay with cAMP-Screen Direct® System.

cAMP-Screen Direct® System, continued

Cyclic AMP immunoassay

Product Configurations

cAMP-Screen Direct® Immunoassay System is available in either a 96-well or 384-well plate format. Each kit format is available in several sizes to provide the ideal-size kit for evaluation, R&D, or screening.

Component	T1505	T1506	T1507	T1508
Plate Format	96-well	384-well	96-well	384-well
Assays per Kit	192	768	960	19,200
Assay/Lysis Buffer	25 mL	65 mL	2 x 65 mL	2 L
cAMP Standard	2 mL	5 mL	2 x 5 mL	100 mL
Anti-cAMP Antibody	14 mL	20 mL	2 x 35 mL	500 mL
cAMP-AP Conjugate	100 mL	250 mL	2 x 250 mL	5 mL
Conjugate Dilution Buffer	10 mL	25 mL	2 x 25 mL	500 mL
Wash Buffer	500 mL (1X)	1 L (1X)	2 x 1 L (1X)	5 x 1 L (5X)
CSPD®/Sapphire-II™ RTU	25 mL	25 mL	2 x 65 mL	650 mLSubstrate/Enhancer Solution
Precoated Plates	2 plates	2 plates	10 plates	50 plates

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
cAMP-Screen Direct® 96-Well Immunoassay System	**192 assays**	**T1505**
cAMP-Screen Direct® 384-Well Immunoassay System	**768 assays**	**T1506**
cAMP-Screen Direct® 96-Well Immunoassay System	**960 assays**	**T1507**
cAMP-Screen Direct® 384-Well Immunoassay System	**19,200 assays**	**T1508**
Assay/Lysis Buffer	**65 mL**	**T2327**
1X Wash Buffer	**1 L**	**T2337**
5X Wash Buffer	**1 L**	**T2356**

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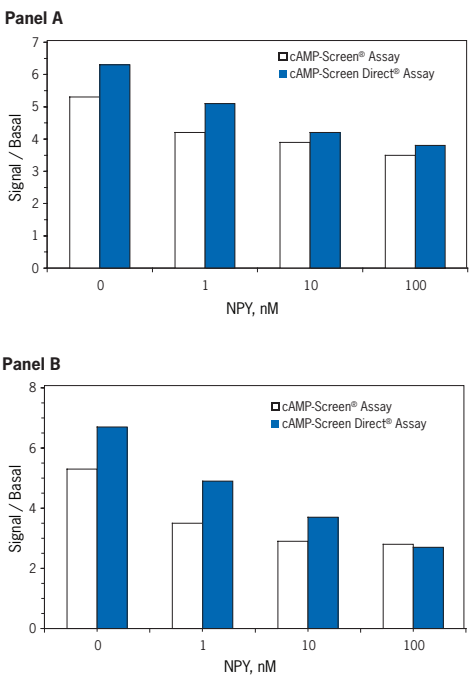

Figure 43. Quantitation of cAMP Levels. Comparison of the cAMP-Screen Direct® System with the cAMP-Screen® System (page 28) for quantitation of cellular cAMP levels. For the cAMP-Screen Direct system, SK-N-MC cells were cultured for four days in cAMP-Screen Direct assay plates and then treated with compounds. The entire assay was performed in the same microplate. For the cAMP-Screen® system, SK-N-MC cells were cultured for four days in a standard microplate and treated with compounds. Cell lysate was transferred to a cAMP-Screen System plate and assayed. **(A)** NPY-mediated inhibition of isoproterenol (10 µM)-stimulated cAMP production. **(B)** NPY-mediated inhibition of forskolin (1 µM)-stimulated cAMP production.

ELISA-Light™ System

Immunoassay

Description

ELISA-Light™ Immunoassay System is used for rapid ultrasensitive antigen detection in enzyme immunoassays employing an alkaline phosphatase label. ELISA-Light system offers an excellent combination of high sensitivity and throughput for assays measured in a luminometer.

ELISA-Light System significantly extends the limits of detection of most immunoassays compared to colorimetric and isotopic methods. The kit contains reagents optimized for chemiluminescent ELISAs employing alkaline phosphatase labels. The kit is available with a choice of Ready-to-Use chemiluminescent substrate formulations containing CSPD® or CDP-*Star*® substrates with a luminescence enhancer (Sapphire-II™ or Emerald-II™ enhancers). I-Block™ blocking reagent, a highly purified casein screened to effectively prevent the nonspecific binding of alkaline phosphatase labeled reagents, antibodies and analytes to solid surfaces, is also included.

Applied Biosystems offers several Ready-to-Use substrate/enhancer formulations optimized for specific assays or luminometers. Formulations containing Sapphire-II enhancer are suitable for most assays performed in tube or microplate luminometers. Formulations containing Emerald-II enhancer are recommended for less-sensitive instruments that require more intense luminescent signals to detect low levels of enzyme. The specific formulation that will provide the highest sensitivity for a specific assay can vary. Therefore an ELISA sampler kit containing all four substrate/enhancer formulations is available to facilitate assay development.

Applications

The ELISA-Light system is used for a variety of protein detection assays, including standard immunoassay formats (see background section), phosphopeptide immunoassays for protein kinase activity quantitation [8,16,22], receptor binding assays [20], and viral foci imaging with immunodetection [11].

ELISA-Light system is also useful for nucleic acid detection applications in microplate-based detection assays. The system reagents are used for highly sensitive DNA probe capture assays, including quantitative detection of labeled PCR products [4] and RNA:DNA hybrids [3,17,21] and immunodetection of chemical-DNA adducts [5].

Product Configuration

ELISA-Light™ System Standard Size

Capacity: 500-1,300 assays
Contents:
- 100 mL of Ready-to-Use chemiluminescent substrate with enhancer
- 100 mL of 10X Assay Buffer
- 7.5 g of I-Block™ blocking reagent

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
ELISA-Light™ Immunoassay System		
Sampler Kit with 25 mL of each formulation listed below (100 mL total)	sampler size	T1022
with 100 mL CSPD® 0.4 mM Ready-to-Use with Sapphire-II™	standard size	T1023
with 100 mL CSPD® 0.4 mM Ready-to-Use with Emerald-II™	standard size	T1024
with 100 mL CDP-*Star*® 0.4 mM Ready-to-Use with Sapphire-II™	standard size	T1025
with 100 mL CDP-*Star*® 0.4 mM Ready-to-Use with Emerald-II™	standard size	T1026
ELISA-Light™ System Accessories available separately, see page 44		
AP-Labeled Secondary Antibody Conjugates		
AP Conjugate, Goat anti-Rabbit IgG	100 µL	T2191
AP Conjugate, Goat Anti-Mouse IgG and IgM	100 µL	T2192
I-Block™ Blocking Reagent	30 g	T2015
Avidx-AP™ Streptavidin-Alkaline Phosphatase Conjugate	1 mL	T2016

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REFERENCES

For a complete list of immunoassay applications with the ELISA-Light™ system and CSPD® and CDP-*Star* substrates please see page 27.

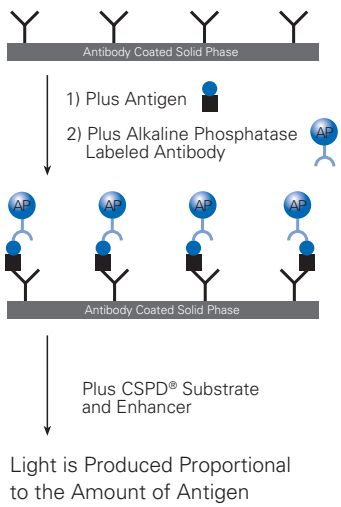


Figure 44. Chemiluminescent Sandwich Immunoassay.

Introduction to Chemiluminescent Membrane-based Immunodetection

Chemiluminescent Immunodetection

Western blotting is a widely used technique for the immunodetection proteins. Protein samples are separated by polyacrylamide gel electrophoresis and electrophoretically transferred to either nitrocellulose, polyvinylidene fluoride (PVDF), or nylon membranes. The resulting blot is incubated with an antigen-specific monoclonal or polyclonal antibody to form a specific antigen-antibody complex. This complex is usually detected with a species-specific secondary antibody, bearing an enzyme label.

Visualization of an enzyme-labeled secondary antibody can be accomplished with colorimetric or chemiluminescent methods. For colorimetric methods, enzyme-catalyzed reactions result in the localized deposition of a colored product on the membrane. The accumulating product often interferes with enzyme activity, limiting sensitivity, and is difficult to remove from the membrane, preventing reuse of the blot. Furthermore, the color usually fades and is difficult to photograph. In addition, colorimetric visualization methods are not quantitative. For chemiluminescent detection, a chemiluminescent substrate solution is added, and the resulting localized light emission is imaged either on X-ray film or with a CCD-based imaging platfrom.

Advantages of Chemiluminescence

Western-Light™ and Western-*Star*™ chemiluminescent Western blotting systems offer highly sensitive, rapid, nonisotopic protein detection [9,12]. Detection is performed on nitrocellulose, nylon, or PVDF membranes. With nitrocellulose membranes, an enhancing agent, Nitro-Block™ or Nitro-Block-II™ enhancer, is added to provide maximum sensitivity. Detection of alkaline phosphatase-labeled immunocomplexes is performed with the CSPD® substrate (Western-Light System; see page 36) or CDP-*Star*® substrate (Western-*Star*™ System; see page 34). Following incubation with substrate, blots are imaged immediately on X-ray film, with exposure times ranging from 30 seconds to 45 minutes with CSPD substrate, and from 1 second to 30 minutes with CDP-*Star* substrate, providing a hard copy of results. CDP-*Star* substrate provides a five- to ten-fold higher signal intensity than CSPD substrate, enabling much shorter exposure times [10]. Detection of alkaline phosphatase labels with 1,2-dioxetane substrates, unlike detection of horseradish peroxidase with enhanced luminol systems, produces a chemiluminescent signal with glow kinetics, enabling multiple exposures over many hours. Glow kinetics, combined with the high intensity signal generated with CDP-*Star* substrate, makes the Western-*Star* detection system ideal for chemiluminescent phosphor screen and CCD camera imaging [17,18].

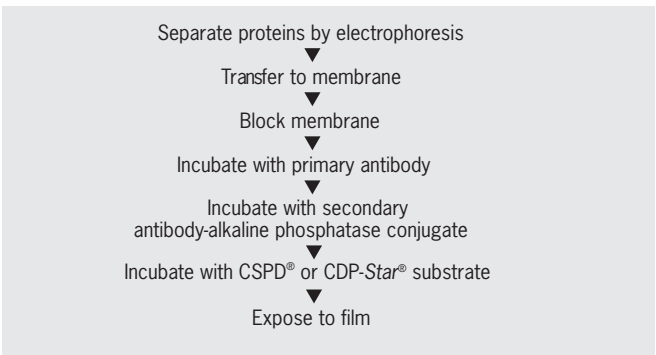
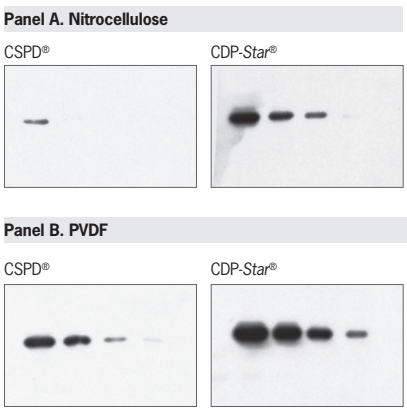


Figure 45. Western-Light™ and Western-*Star*™ Systems Immunodetection Procedure.



(A) Human brain extract (10, 5, 2.5, 1.25 µg) was electrophoretically separated and transferred to Tropifluor™ PVDF and nitrocellulose membrane. **(B)** Blots were detected with the Western-Light™ system and CSPD® substrate or the Western-*Star*® system and CDP-*Star*® substrate, with enhancer for nitrocellulose and without enhancer for PVDF. Blots were imaged on Kodak XAR-5 film for 30 sec, 20 min after substrate incubation.

Figure 46. Detection with CDP-*Star*® Substrate vs. CSPD® Substrate.

Biotinylated Secondary Antibodies and Proteins

Immunoblotting can be performed with a biotinylated secondary antibody followed by streptavidin-alkaline phosphatase conjugate and CSPD or CDP-*Star* substrate, and often results in increased signal intensity with shorter exposure times compared to that obtained with alkaline phosphatase conjugated secondary antibody. Tropix® chemiluminescent substrates have been used for highly sensitive detection of biotin-labeled proteins [12]. Other applications include detection of immunoprecipitated biotin labeled proteins [11,13,15] and biotinylated peptide probes.

Detection of Phosphorylated Proteins

Detection of phosphotyrosine or phosphoserine and phosphothreonine-containing proteins on immunoblots has been widely used for the analysis of protein kinase substrates and activities [14,16]. Chemiluminescent immunoblot detection of phosphorylated proteins can be performed with the Western-*Star* or Western-Light chemiluminescent detection systems in conjunction with the appropriate anti-phospho-epitope primary antibody. A non-casein blocker should be substituted for the I-Block™ blocking reagent, since it is casein and contains phosphorylated amino acids.

Detection System	Western-*Star*™ see page 392	Western-Light™ see page 394
Key Attribute	More sensitive than Western-Light™	Established system with good sensitivity
Substrate	CDP-*Star*®	CSPD®
Detection Scheme	Alkaline Phosphatase-2° Ab Conjugate	Alkaline Phosphatase-2° Ab Conjugate
Film Exposure Time	1 sec - 30 min	2 min - 45 min
Special Features	Highest signal intensity—ideal for CCD camera and phosphor screen imaging	Original chemiluminescent Western system

Figure 47. Selection Guide for Western Blot Detection Systems. Applied Biosystems offers two Western blot detection systems.

REFERENCES

See Western-Light™ and Western-*Star*™ Immunodetection System sections (pages 35 and 37) for references.

Western-*Star*™ System

Immunodetection

Description

Western-*Star*™ Immunodetection System is a highly sensitive chemiluminescent immunodetection system that provides speed, flexibility, control of film exposure time and the ability to use alternative imaging systems. Western-*Star* system incorporates CDP-*Star*® substrate for detection of secondary antibody-alkaline phosphatase conjugates.

Advantages

Western-*Star* system generates a high intensity chemiluminescent signal that persists from hours to days, depending on the membrane type. CDP-*Star* substrate provides a five- to tenfold higher signal intensity than CSPD® substrate. Images can be generated immediately on X-ray or instant film to provide permanent, hard-copy results. In addition, the high signal intensity and long-lived signal generated with CDP-*Star* substrate provides an ideal quantitative detection system for chemiluminescent phosphor screen and CCD camera imaging systems.

Applications

Western-*Star* Immunodetection System is used for highly sensitive immunoblot detection of proteins, biotinylated proteins [1], and phosphoproteins in protein extracts from many sources, including cell cultures and tissues. These reagents are compatible with multiple types of membranes, including PVDF, nylon and nitrocellulose. Detection is performed directly with AP-labeled secondary antibodies, or with indirect detection of biotinylated antibodies or biotinylated proteins with a streptavidin-AP conjugate (Avidx-AP™, see Reagents and Accessories section, page 44). Western-*Star* System has been used for confirmation of protein knockdown for siRNA gene expression regulation experiments [4].

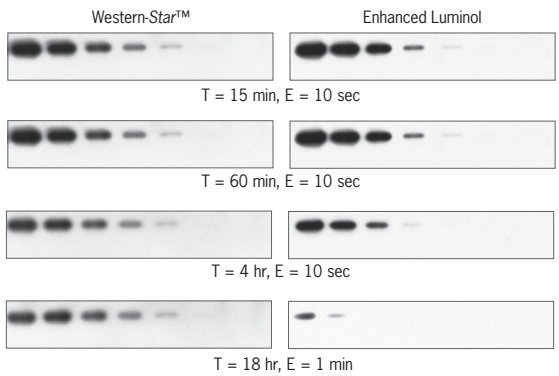


Figure 48. Western-*Star*™ System vs. Enhanced Luminol Detection. Human brain extract was electrophoretically separated and transferred to PVDF. Blots were incubated with a monoclonal anti-actin, followed by Western-*Star*™ detection or with a horseradish peroxidase (HRP)-conjugated secondary antibody, followed by enhanced luminol substrate detection. Blots were imaged on Kodak XAR-5 X-ray film. T = time after substrate incubation and E = exposure time.

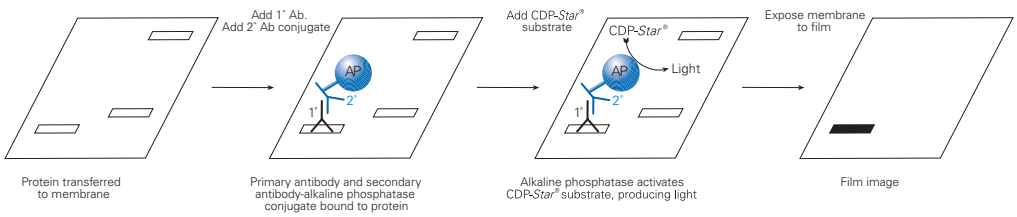


Figure 49. Immunodetection with Western-*Star*™ System.

Western-*Star*™ System, continued

Immunodetection

Product Configuration

Western-*Star*™ System Standard Size

T1048
with Goat Anti-Rabbit IgG AP Conjugate
T1046
with Goat Anti-Mouse IgG+IgM AP Conjugate
Capacity: 30 membrane blots (10 cm x 10 cm)
Contents:
- 100 mL CDP-*Star*® 0.25 mM Ready-to-Use substrate
- secondary antibody AP conjugate
- 30 g I-Block™ blocking reagent
- 5 mL Nitro-Block-II™ chemiluminescence enhancer
- 150 mL 10X Assay Buffer concentrate
- 30 development folders (14 cm x 19 cm)

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Western-*Star*™ System		
with Goat Anti-Mouse IgG+IgM AP Conjugate	standard size	T1046
with Goat Anti-Rabbit IgG AP Conjugate	standard size	T1048
Western-*Star*™ System Accessories	(available separately, see page 44)	
Goat Anti-Rabbit IgG AP Conjugate	100 µL	T2191
Goat Anti-Mouse IgG+IgM AP Conjugate	100 µL	T2192
I-Block™ Blocking Reagent	30 g	T2015
Tropifluor™ PVDF Membrane (15 cm x 15 cm)— 5 Membranes/Pack	1 pack	T2234
Development Folders (14 cm x 19 cm)— 30 Folders/Pack	1 pack	T2258

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REFERENCES

(For a complete Western-*Star*™ immunodetection system reference list, please see the Applied Biosystems website.)

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Western-Light™ System

Immunodetection

Description

Western-Light™ System is a versatile non-isotopic, chemiluminescent immunodetection system that provides speed, sensitivity, flexibility, and control of film exposures. Western-Light System incorporates high-performance CSPD® substrate for detection of a secondary antibody-alkaline phosphatase conjugate.

Advantages

Western-Light System detection generates a chemiluminescent signal that persists from hours to days, depending on the type of membrane used. This enables an image to be generated on X-ray or instant film to provide a permanent hard copy of results. Multiple images may be rapidly and easily acquired with excellent control over film exposures. Detection of sub-picogram levels of protein is easily attainable (the ultimate sensitivity depends on primary antibody quality). New users of 1,2-dioxetane substrates for Western blot detection are recommended to use the Western-*Star*™ immunodetection system for maximum signal intensity and sensitivity.

Applications

Western-Light immunodetection system is used for highly sensitive immunoblot detection of proteins, biotinylated proteins, and phosphoproteins in protein extracts from many sources, including cell cultures and tissues. These reagents are compatible with multiple types of membranes, including PVDF, nylon and nitrocellulose. Detection is performed directly with AP-labeled secondary antibodies, or with indirect detection of biotinylated antibodies or biotinylated proteins with a streptavidin-AP conjugate (Avid*x*-AP™, see Reagents and Accessories section, page 44).

Product Configuration

Western-Light™ System Standard Size

T1047 with Goat Anti-Rabbit IgG AP Conjugate
T1045 with Goat Anti-Mouse IgG+IgM AP Conjugate
Capacity: 30 membrane blots (10 cm x 10 cm)
Contents:
- 100 mL CSPD® 0.25 mM Ready-to-Use substrate
- secondary antibody AP conjugate
- 30 g I-Block™ blocking reagent
- 5 mL Nitro-Block™ chemiluminescence enhancer
- 150 mL 10X Assay Buffer concentrate
- 30 development folders (14 cm x 19 cm)

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Western-Light™ System		
with Goat Anti-Mouse IgG+IgM AP Conjugate	standard size	T1045
with Goat Anti-Rabbit IgG AP Conjugate	standard size	T1047
Western-Light™ System Accessories (available separately, see page 44)		
Goat Anti-Rabbit IgG-AP Conjugate	100 µL	T2191
Goat Anti-Mouse IgG+IgM AP Conjugate	100 µL	T2192
I-Block™ Blocking Reagent	30 g	T2015
Tropifluor™ PVDF Membrane (15 cm x 15 cm)— 5 Membranes/Pack	1 pack	T2234
Development Folders (14 cm x 19 cm)— 30 Folders/Pack	1 pack	T2258

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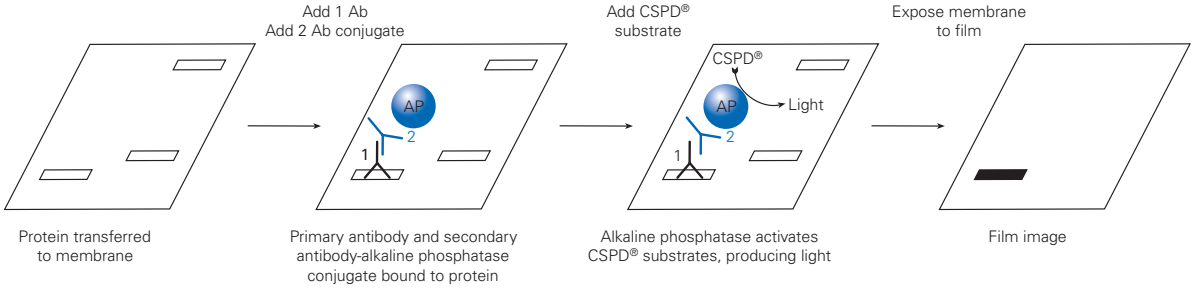
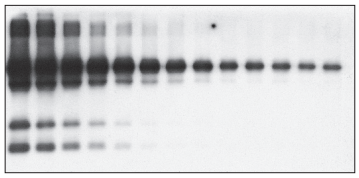


Figure 50. Immunodetection with Western-Light™ System.

Western-Light™ System, continued

Immunodetection

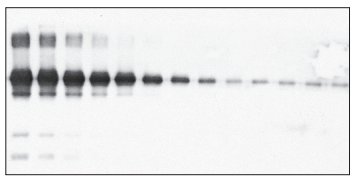
Panel A. Tropifluor™ PVDF



15 7.5 3.8 1.9 0.9 0.5 0.2 0.1 .06 .03 .015 .007 .004

(ng)

Panel B. Nitrocellulose (with Nitro-Block™ enhancer)



15 7.5 3.8 1.9 0.9 0.5 0.2 0.1 .06 .03 .015 .007 .004

(ng)

Figure 51. Western-Light™ Immunodetection with Tropifluor™ PVDF vs. Nitrocellulose. Purified human transferrin was electrophoretically separated and transferred to membrane, incubated with rabbit anti-transferrin polyclonal antibody, detected using Western-Light™ system, and exposed immediately on X-ray film for 15 minutes. As shown, less than 3.6 pg of protein is detectable on both Tropifluor PVDF membrane (without Nitro-Block enhancer treatment) and nitrocellulose membrane (with Nitro-Block™ enhancer treatment).

REFERENCES

(For a complete Western-Light™ immunodetection system reference list, please see the Applied Biosystems website.)

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4
Nucleic Acid Membrane-Based Detection Products

Introduction

Chemiluminescent Nucleic Acid Detection

Traditional nucleic acid detection methods employ radioisotopes such as ^{32}P, which have several disadvantages. Exposures often require several days and labeled probes must be prepared frequently because of radiolytic decay. Furthermore, the radiation safety precautions required are inconvenient and disposal costs are continually increasing.

Applied Biosystems has developed non-isotopic chemiluminescent technologies for detecting nucleic acids utilizing enzyme conjugates, such as alkaline phosphatase conjugates or alkaline phosphatase-labeled DNA probes. As shown, alkaline phosphatase activates the chemiluminescent CDP-*Star*® substrate to produce light. A distinct band is formed since dephosphorylated CDP-*Star* substrate has a strong affinity for hydrophobic sites of the membrane. The localized persistent "glow" of light can be imaged on X-ray or instant film or with CCD camera-based imaging systems. Film exposures of 5 to 60 minutes are easily performed and sub-picogram quantities of labeled DNA can be detected.

Indirect Detection

Nucleic acid detection with CDP-*Star* or CSPD® chemiluminescent substrate is performed with either a direct or indirect alkaline phosphatase label with Southern-*Star*™ or Southern-Light™ systems. Indirect detection of biotin-labeled probes is accomplished with a streptavidin-alkaline phosphatase conjugate (Avidx-AP™, see page 44). This conjugate is optimized to exhibit minimum nonspecific binding and superior sensitivity. Detection of other hapten labels, such as digoxigenin, fluorescein or DNP, with the appropriate antibody-alkaline phosphatase conjugate, can also be performed successfully.

Alkaline Phosphatase-oligonucleotide Conjugates

The use of oligonucleotides covalently derivatized with alkaline phosphatase is currently the most effective method for exploiting the inherent sensitivity of dioxetane chemiluminescence. This direct method offers high sensitivity, low background, short hybridizations, and relatively rapid film exposures [20]. A single copy gene can be detected in 0.25 µg of human genomic DNA using an alkaline phosphatase-derivatized oligonucleotide [6,17]. Use of CDP-*Star* substrate reduces exposure times from greater than 12 hours to less than 2 hours [17] in many applications.

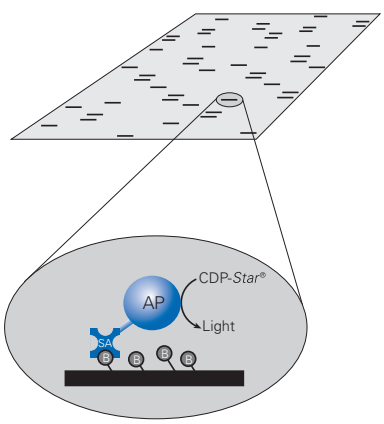
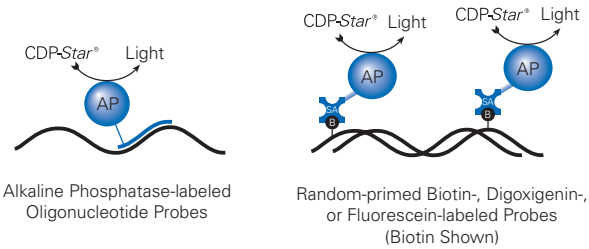


Figure 52. Detection of Biotin-Labeled DNA with CDP-*Star*® Substrate.



Alkaline Phosphatase-labeled Oligonucleotide Probes

Random-primed Biotin-, Digoxigenin-, or Fluorescein-labeled Probes (Biotin Shown)

Figure 53. Nucleic Acid Labeling Methods for Chemiluminescent Detection.

1,2-Dioxetane chemiluminescent substrates, including CDP-*Star* and CSPD substrates, have also been successfully utilized in sensitive, non-radioactive detection in a variety of membrane-based nucleic acid detection methods, including:

- Northern blotting [3,13,15,18]
- RNA knockdown detection [10]
- capped mRNA detection [14]
- DNA macroarray hybridization for DNA analysis [12] and gene expression analysis [19]
- direct detection of PCR-amplified DNA [2,9]
- SSCP analysis [21], differential display [1]
- plaque hybridization
- gel shift assays [8]
- RNase protection assays [11]
- RNA probe detection of RNA binding proteins [22]

1,2-Dioxetane chemiluminescent substrates have also been employed in a chemiluminescent in situ hybridization assay with DIG-labeled probes [16].

Introduction, continued

Detection System	Key Attribute	Substrate	Detection Scheme	Film Exposure Time	Special Features
Southern-*Star*™ for detection of biotin-labeled probes see page 41	Most sensitive system for biotin detection	CDP-*Star*®	Streptavidin-AP Conjugate (Avidx-AP™)	1 min–2 hr	Compared to ^{32}P, reduces overnight film exposures to less than 2 hr. Applications include: Southern and Northern blotting, colony/plaque screening, and direct detection of AP-oligo probes
Southern-Light™ for detection of biotin-labeled probes see page 42	Established system with good sensitivity	CSPD®	Streptavidin-AP Conjugate (Avidx-AP™)	5 min–10 hr	Original system for chemiluminescent Southern blotting. Applications include Southern and Northern blotting, colony/plaque screening, and direct detection of AP-oligo probes.

Figure 54. Selection Guide for Southern/Northern Blot Detection Systems. Applied Biosystems offers several Southern blotting systems. High sensitivity in non-radioactive Southern blots is achieved by efficient probe labeling and following proper blocking and wash steps as described in protocols. The chart below summarizes the differences and relative merits of each system. Southern-*Star*® system, which provides the highest signal intensity and sensitivty, is recommended to new users for all applications.

REFERENCES

(For a complete Southern-Light™ and Southern-*Star*™ nucleic acid detection reference lists, please see the Applied Biosystem website.)

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Southern-*Star*™ System

Nucleic acid detection system for biotin-labeled probes

Description

Southern-*Star*™ nucleic acid detection system is a non-isotopic, chemiluminescence-based products for detection of biotin-labeled nucleic acids in Southern and Northern blotting with high sensitivity and ease of use. Southern-*Star* detection of biotin-labeled nucleic acid probes incorporates CDP-*Star*® substrate and Avidx-AP™ streptavidin-alkaline phosphatase conjugate. Southern-*Star* system reagents are compatible with other labeling systems, including digoxigenin, fluorescein and DNP with the appropriate antibody-alkaline phosphatase conjugates, as well as alkaline phosphatase-labeled oligonucleotides.

Advantages

CDP-*Star* substrates the chemiluminescent substrate included in Southern-*Star* system, generates a high intensity chemiluminescent signal that may persist for 2-3 days on nylon membrane, allowing multiple images to be acquired. CDP-*Star* substrate provides up to a ten-fold higher signal intensity than CSPD® substrate (included with the Southern-Light™ system (Figure 55), and reaches maximum light level more rapidly. Images can be obtained immediately on X-ray or instant film to provide permanent, hard-copy records. In addition, the high signal intensity and long duration produced by CDP-*Star* substrate makes Southern-*Star* system detection ideal for chemiluminescent phosphor screen and CCD camera imaging systems.

Applications

Southern-*Star* nucleic acid detection system is used for highly sensitive detection of DNA for Southern blotting applications [7], including DNA detection and gene expression analysis on DNA microarrays [12,19] and direct detection of labeled DNA on membranes. It can also be used for additional nucleic acid detection applications, including colony and plaque screening, Northern blotting [3,15], RNA knockdown analysis [10], capped mRNA detection [14], RNase protection, and gel-shift assays.

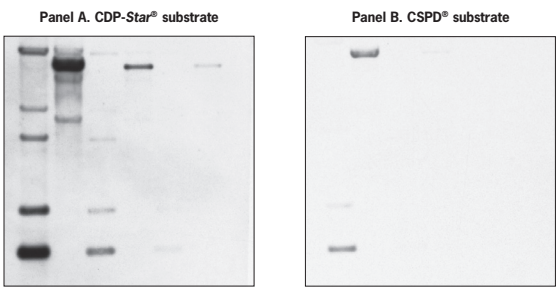

Figure 55. Southern-*Star*™ Nucleic Acid Detection with CDP-*Star*® vs. CSPD® Substrates. Yeast *S. cerevisiae* genomic DNA (EcoRI and Bgl II digested) was electrophoretically separated and transferred to nylon membrane. Blots were hybridized with a single copy RPB 1 probe and detected with Avidx-AP™ conjugate and 0.25 mM CSPD® **(Panel A)** or CDP-*Star*® **(Panel B)** substrate, according to the Southern-*Star*™ system protocol. Blots were incubated with substrate for 75 min and then exposed to X-ray film for 4 min.

Product Configuration

Southern-*Star*™ System Standard Size

T1040
Capacity: 30 membrane blots (10 cm x 10 cm)
Contents:
- 100 mL CDP-*Star*® 0.25 mM Ready-to-Use substrate
- 120 µL Avidx-AP™ streptavidin-alkaline phosphatase conjugate
- 30 g I-Block™ blocking reagent
- 150 mL 10X Assay Buffer concentrate
- 30 development folders (14 cm x 19 cm)

T1039
T1040 System with Tropilon-Plus™ Membrane

Southern-*Star*™ System Small Size

T1042
Capacity: 10 membrane blots (10 cm x 10 cm)
Contents:
- 30 mL CDP-*Star*® 0.25 mM Ready-to-Use substrate
- 40 µL Avidx-AP™ streptavidin-alkaline phosphatase conjugate
- 7.5 g I-Block™ blocking reagent
- 50 mL 10X Assay Buffer concentrate
- 10 development folders (14 cm x 19 cm)

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Southern-*Star*™ Nucleic Acid Detection System for Biotin-Labeled Probes		
with Tropilon-Plus™ Membrane	standard size, 30 blots	T1039
	standard size, 30 blots	T1040
	small size, 10 blots	T1042
Southern-*Star*™ System Accessories (available separately, see page 44)		
I-Block™ Blocking Reagent	30 g	T2015
Avidx-AP™ Streptavidin-AP Conjugate	1 mL	T2016
Tropilon-Plus™ Nylon Membrane (30 cm x 200 cm)	1 roll	T2232

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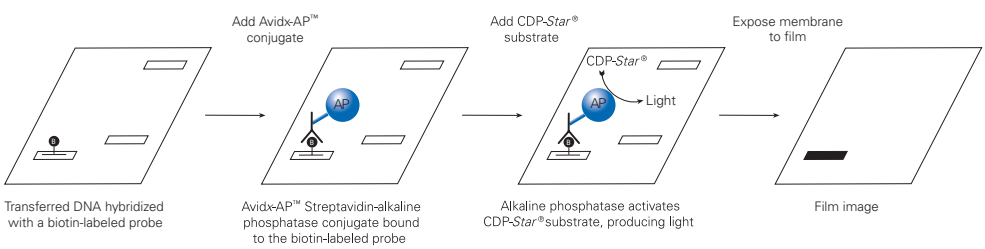

Figure 56. Nucleic Acid Detection with Southern-*Star*™ System.

Southern-Light™ System

Nucleic acid detection system for biotin-labeled probes

Description

Southern-Light™ nucleic acid detection system is a non-isotopic, chemiluminescence-based product for detection of biotin-labeled nucleic acids in Southern and Northern blotting with high sensitivity and ease of use. Southern-Light detection of biotin-labeled nucleic acid probes incorporates CSPD® substrate and Avidx-AP™ streptavidin-alkaline phosphatase conjugate. Southern-Light system reagents are compatible with other labeling systems, including digoxigenin, fluorescein and DNP with the appropriate antibody-alkaline phosphatase conjugates, as well as alkaline phosphatase-labeled oligonucleotides.

Advantages

The convenience, speed, sensitivity, and versatility of chemiluminescent Southern blotting makes radioactive methods obsolete. Each component of the Southern-Light system is optimized for maximum signal intensity and low background, including CSPD high-performance chemiluminescent alkaline phosphatase substrate, Avidx-AP streptavidin-alkaline phosphatase conjugate, and I-Block™ blocking reagent. The Southern-Light system procedure is able to detect less than 1 pg of target DNA. Most exposures require less than 60 minutes and are performed with standard X-ray or instant film. Light emission persists for days enabling multiple re-exposures. Additionally, membranes are easily stripped and reprobed.

Applications

Southern-Light nucleic acid detection system is used for highly sensitive detection of DNA for Southern blotting applications and direct detection of labeled DNA on membranes. This system can also be used for additional nucleic acid detection applications, including colony and plaque screening, Northern blotting, RNase protection, and gel-shift assays. The Southern-Light system is also useful for detection of biotinylated proteins in non-isotopic immunoprecipitation procedures.

Product Configuration

Southern-Light™ System Standard Size

T1037
Capacity: 30 membrane blots (10 cm x 10 cm)
Contents:
- 100 mL CSPD® 0.25 mM Ready-to-Use substrate
- 120 µL Avidx-AP™ streptavidin-alkaline phosphatase conjugate
- 30 g I-Block™ blocking reagent
- 150 mL 10X Assay Buffer concentrate
- 30 development folders (14 cm x 19 cm)

T1038
T1037 System with Tropilon-Plus™ Membrane

Southern-Light™ System Small Size

T1041
Capacity: 10 membrane blots (10 cm x 10 cm)
Contents:
- 30 mL CSPD® 0.25 mM Ready-to-Use substrate
- 40 µL Avidx-AP™ streptavidin-alkaline phosphatase conjugate
- 7.5 g I-Block™ blocking reagent
- 50 mL 10X Assay Buffer concentrate
- 10 development folders (14 cm x 19 cm)

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
Southern-Light™ Nucleic Acid Detection System for Biotin-Labeled Probes		
	standard size, 30 blots	T1037
with Tropilon-Plus™ Membrane	standard size, 30 blots	T1038
	small size, 10 blots	T1041
Southern-Light™ Accessories (available separately, see page 44)		
I-Block™ Blocking Reagent	30 g	T2015
Avidx-AP™ Streptavidin-AP Conjugate	1 mL	T2016
Tropilon-Plus™ Nylon Membrane (30 cm x 200 cm)	1 roll	T2232

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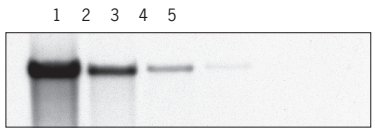

Figure 57. Nucleic Acid Detection with Fluorescein-Labeled Probe. Southern-Light™ system detection of *S. cerevisiae* single copy RNA polymerase gene RPB1 using a fluorescein-labeled probe. Lanes 1 through 5 contain 2 µg, 400 ng, 80 ng, 16 ng, and 3.2 ng total DNA, respectively.

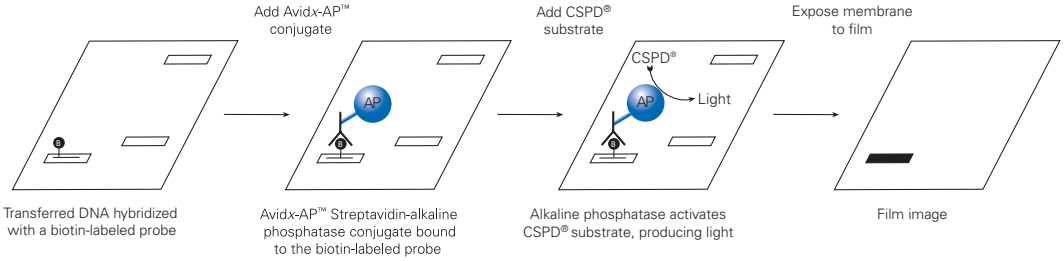

Figure 58. Nucleic Acid Detection with Southern-Light™ System.

5
Reagents and Accessories for Chemiluminescence

Reagents and Accessories for Chemiluminescence

AP-Labeled Secondary Antibody Conjugates

Secondary antibody-alkaline phosphatase conjugates are optimized for Western blotting and immunoassay procedures incorporating 1,2-dioxetane enzyme substrates. These labeled secondary antibodies provide minimum nonspecific binding and are recommended for applications employing Tropix chemiluminescent substrates. The conjugates are supplied at approx. 0.3 mg/mL in a buffered solution containing 50% glycerol. Goat anti-rabbit IgG (H+L) exhibits minimum cross-reactivity with human serum proteins. Goat anti-mouse IgG+IgM (H+L) exhibits minimum cross-reactivity with human, bovine, and horse serum proteins. The recommended initial working dilution for these labeled antibodies is 1:5,000; the best working dilution may need to be optimized for different uses.

Avidx-AP™ Streptavidin-AP Conjugate

Avidx-AP™ conjugate is a streptavidin-alkaline phosphatase conjugate developed for detection of biotinylated nucleic acids or proteins. The conjugate is tested and optimized for low background with chemiluminescent detection procedures. Conjugation methods developed at Applied Biosystems result in minimal nonspecific binding of conjugate to membranes. Avidx-AP conjugate is available as part of Southern-Light™ and Southern-*Star*™ detection systems and is also sold separately, for use with other applications, such as immunoassay detection with biotinylated antibodies, microplate detection assays for nucleic acids labeled with biotin, or other assays that incorporate a biotin label. The suggested working dilution of Avidx-AP conjugate is from 1:5,000 to 1:20,000.

DEA (Diethanolamine) Buffer Concentrate

DEA is the buffer of choice in applications employing concentrated Tropix® substrates for the detection of alkaline phosphatase. DEA buffers provide favorable reaction kinetics and greater sensitivity compared to most buffer systems. DEA is supplied 99% pure (MW = 105.1 g/mol; density = 1.097 g/mL). The suggested working concentration is 0.1 M DEA (pH 10) with 1 mM $MgCl_2$.

I-Block™ Protein-based Blocking Reagent

I-Block™ reagent is a highly purified casein-based blocking reagent. The reagent provides superior blocking compared to both dried milk and BSA. Unlike other casein-based blocking reagents, I-Block reagent is essentially biotin-free. I-Block reagent is tested in assays using Tropix substrates and alkaline phosphatase conjugates, and is useful as a blocking reagent in membrane-based and immunoassay applications. The suggested working concentration is 0.2% (w/v) for detection of nucleic acids on neutral or positively-charged nylon membranes, and for immunoassays and protein detection on membranes (nitrocellulose, PVDF, or neutral nylon). For protein detection on positively-charged nylon membrane (such as Tropilon-Plus™ membrane), a concentration of 3% is recommended. I-Block blocking solution is prepared in either Tris- or phosphate-buffered saline buffer with heating (40-50°C).

Tropifluor™ Polyvinylidene Fluoride (PVDF) Membrane

Tropifluor™ PVDF transfer membrane is a 0.45 µm pore-size membrane for protein blotting that provides the high binding (125 µg/cm^2) and retention capacity inherent in PVDF (polyvinylidene fluoride) membranes. The low background and resulting superior signal-to-noise performance obtained with Tropifluor membrane enables picogram detection levels of proteins in immunoblotting applications. Tropifluor membrane is selected to yield low background levels with the use of Tropix substrates and conjugates, enabling ultrasensitive detection. PVDF membranes exhibit high mechanical strength that enable multiple re-probing. Film exposures using Tropix substrates with Tropifluor membrane range from 1 second to 15 minutes. The combination of chemiluminescent detection with Western-Light™ or Western-*Star*™ immunoblotting detection systems and the high protein-binding capacity of Tropifluor membrane provides the greatest sensitivity available in Western blotting.

Tropilon-Plus™ Positively Charged Nylon Membrane

Tropilon-Plus™ nylon membrane is a 0.45 µm pore-size, positively-charged membrane for nucleic acid blotting. The inherent positive charge offers increased binding capacity (>100 µg/cm^2) and signal retention in nucleic acid detection experiments. In addition, Tropilon-Plus membrane has excellent lot-to-lot consistency with low within-lot binding capacity variances.

Tropilon-Plus membrane is optimized for chemiluminescent detection using Tropix substrates and alkaline phosphatase conjugates. Background levels associated with chemiluminescent methods can vary dramatically with other nylon membranes. Tropilon-Plus membrane provides a high-intensity signal with low levels of nonspecific background.

Tropilon-Plus membrane offers several advantages compared to neutral nylon membrane and other types of positively-charged nylon membranes. DNA binding capacity is higher and background signal is lower using the Tropilon-Plus membrane.

APPLIED BIOSYSTEMS ORDER INFO	SIZE	CAT#
AP-Conjugate - Goat Anti-Rabbit IgG	100 µL	T2191
AP-Conjugate - Goat Anti-Mouse IgG+IgM	100 µL	T2192
Avidx-AP™ Streptavidin-AP Conjugate	1 mL	T2016
DEA (Diethanolamine) Buffer Concentrate	120 µL	T2027
I-Block™ Blocking Reagent	30 g	T2015
Tropifluor™ PVDF Membrane (15 cm x 15 cm) - 5 Membranes/Pack	1 pack	T2234
Tropilon-Plus™ Nylon Membrane (30 cm x 200 cm)	1 roll	T2232
Development Folders (14 cm x 19 cm) - 30 Folders/Pack	1 pack	T2258
Assay Buffer (10X) for Immunoassay, Western, and Southern Blotting	150 mL	T2187

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