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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; Glucostar® 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.

Introduction

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Substrates and Enhancers

**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 placent al alkaline phosphatase (PLAP) and secreted placent al alkaline phosphatase (SEAP) in reporter gene assays [6,12]. 1,2-Dioxetane substrates for alkaline phosphatase are used widely in immunoblotting 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**

Glucostar™ substrate is utilized for highly sensitive detection of β-glucosidase. The use of Glucostar 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 luminal, 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**

CSPD-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.)**


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 immunooassays, 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 immunooassay 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 proteinase 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).

REFERENCES

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

**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 immunoassay detection 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.
**Glucuron® 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].

**REFERENCES**

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

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**Glucon™ 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.

**REFERENCES**

**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.

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Substrates and Enhancers

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 quantify 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.

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 reagents, and microplates everything needed for fast, accurate quantitation of neuraminidase inhibitor resistance in influenza virus isolates.

REFERENCES

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Substrates and Enhancers

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).

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 Variety Pack

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Enhancer Variety Pack

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References

Substrates and Enhancers

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.

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.

Nitrocellulose

without Nitro-Block™

with Nitro-Block™

PVDF

without Nitro-Block™

with Nitro-Block™

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

Figure 13. Enhancer Comparison for Membrane-based Assays.

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Reporter Gene Assays and Reagents

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Reported Gene Assays and Reagents

Introduction

Reported Gene Assays

Reported 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 typically 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 substrates for β-galactosidase, the sensitivity is increased dramatically [3,4].

<table>
<thead>
<tr>
<th>Reporter Gene</th>
<th>Detection Method</th>
<th>Detection Limit</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol Acetyl Transferase (CAT)</td>
<td>Isotopic ELISA</td>
<td>5x10^5 molecules 1x10^6 molecules</td>
<td>Widely Used No Radioactivity</td>
<td>Radioactive High Cost Low Dynamic Range Labor Intensive Low Dynamic Range High Cost/Assay Labor Intensive</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>ONPG (Color) MUG (Fluorescence) GaLScreen System GalactoStar System Galacto Light Plus System and DualLight System (Luminescence)</td>
<td>3x10^7 molecules 6x10^7 molecules 3x10^8 molecules</td>
<td>Widely Used High Sensitivity Wide Dynamic Range Simplicity</td>
<td>Poor Sensitivity Auto-fluorescence</td>
</tr>
<tr>
<td>Human Growth Hormone</td>
<td>Radioimmunoassay</td>
<td>3x10^7 molecules</td>
<td>Secreted into Media</td>
<td>Radioactivity High Cost/Assay Low Sensitivity</td>
</tr>
<tr>
<td>Luciferase</td>
<td>Luciplex System Tropix Luciferase Assay Kit DualLight System (Luminescence)</td>
<td>10^7-10^8 molecules</td>
<td>Assay Simplicity High Sensitivity Wide Dynamic Range</td>
<td>Protein Instability</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>MUG (Fluorescence) Glucuron® Substrate Luminescence</td>
<td>2x10^7 molecules 5x10^7 molecules</td>
<td>Autofluorescence High Sensitivity Simplicity Wide Dynamic Range</td>
<td>Protein Quenching</td>
</tr>
<tr>
<td>Secreted Placental Alkaline Phosphatase</td>
<td>pNPP (Color) PhosphaLight System Luminescence</td>
<td>1x10^7 molecules 3x10^8 molecules</td>
<td>Secreted into Media High Sensitivity Wide Dynamic Range</td>
<td>Poor Sensitivity</td>
</tr>
</tbody>
</table>

Luciferase has become increasingly popular as a reporter gene, especially for co-transfection experiments where it is important to normalize transfection efficiency. Assays with limited dynamic range, such as CAT, require testing of several sample dilutions to verify that sample values are within the linear range.

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.

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**


---

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

---

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.
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**
  - 0.8 mL Galacton-Star® Substrate
- **T1032 – Reaction Buffer B**
  - 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**
  - 4 mL Galacton-Star® Substrate
- **T1030 – Reaction Buffer B**
  - 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**
  - 42 mL Galacton-Star® Substrate
- **T1031 – Reaction Buffer B**
  - 1,000 assays T1027 or 1,000 assays T1030 or 200 assays T1032

**Gal-Screen® System for Yeast or Mammalian Cells**

- **T1032 – Reaction Buffer B**
  - 4 mL Galacton-Star® Substrate
  - 100 assays T1027 or 100 assays T1030 or 100 assays T1031

**Gal-Screen® System for Yeast or Mammalian Cells**

- **T1029 – Reaction Buffer A**
  - 0.8 mL Galacton-Star® Substrate

**APPLIED BIOSYSTEMS ORDER INFO**

<table>
<thead>
<tr>
<th>SIZE</th>
<th>CAT#</th>
</tr>
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<tr>
<td>200 assays</td>
<td>T1029</td>
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<tr>
<td>1,000 assays</td>
<td>T1027</td>
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<tr>
<td>10,000 assays</td>
<td>T1028</td>
</tr>
<tr>
<td>200 assays</td>
<td>T1032</td>
</tr>
<tr>
<td>1,000 assays</td>
<td>T1030</td>
</tr>
<tr>
<td>10,000 assays</td>
<td>T1031</td>
</tr>
</tbody>
</table>

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


Learn more or order the products on this page at www.appliedbiosystems.com
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 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]. Galacto-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.

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**Galacto-Star™ System Assay System, continued**

**One-Step β-galactosidase reporter gene assay**

### Product Configuration

**Galacto-Star™ System Standard Size**

- **T1012** – Mammalian Cells
  - Capacity: 200 single tube assays
  - Content: 1.2 mL Galacto-Star® substrate
- **T1019** – Yeast Cells
  - Capacity: 600 assays with microplate format
  - Content: 60 mL Reaction Buffer Diluent with SapphiRe® enhancer
  - 80 mL Mammalian Lysis Solution or 240 mL 5X Yeast Lysis Buffer

**Galacto-Star™ System Large Size**

- **T1014** – Mammalian Cells
  - Capacity: 600 single tube assays
  - Content: 1.800 assays with microplate format
  - Content: 3.6 mL Galacto-Star® substrate
  - 180 mL Reaction Buffer Diluent containing SapphiRe® enhancer
  - 210 mL Mammalian Lysis Solution or 240 mL 5X Yeast Lysis Buffer

**Galacto-Star™ System Screening Size**

- **T1013** – Mammalian Cells
  - Capacity: 15,000 assays with microplate format
  - Content: 30 mL Galacto-Star® substrate
  - 1.5 L Reaction Buffer Diluent containing SapphiRe® enhancer
  - 1.75 L Mammalian Lysis Solution or 2 L 5X Yeast Lysis Buffer

### Applied Biosystems Order Info

- **Galacto-Star™ Mammalian Cell Reporter Gene Assay System**
  - Standard size: T1012
  - Large size: T1014
  - Screening size: T1013
- **Galacto-Star™ Yeast Cell Reporter Gene Assay System**
  - Standard size: T1019
  - Large size: T1021
- **Galacto-Star™ Yeast Cell Reporter Assay System**
  - Screening size: T1020
- **Galacto-Star™ Reaction Buffer Diluent with Galacto-Star® Chemiluminescent Substrate**
  - 180 mL: 1.7 mL T1056
  - 3.6 mL: T1056

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Reported Gene Assays and Reagents

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

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 include Galacton® chemiluminescent β-galactosidase substrate and require the use of a luminometer equipped with an injector. Galacto-Light Plus Assay systems include 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 lysis 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 [25] 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.](image)

![Figure 24. Detection of β-Galactosidase with Galacto-Light™ System.](image)

![Figure 25. β-Galactosidase Reporter Gene Assay with Galacto-Light Plus™ System.](image)
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.

<table>
<thead>
<tr>
<th>Product Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Galacto-Light™ or Galacto-Light Plus™ System Standard Size</strong></td>
</tr>
<tr>
<td>T1006 – Galacto-Light™ System</td>
</tr>
<tr>
<td>T1007 – Galacto-Light Plus™ System</td>
</tr>
<tr>
<td>Capacity: 200 single tube assays</td>
</tr>
<tr>
<td>Capacity: 600 assays with microplate format</td>
</tr>
<tr>
<td>Contents:</td>
</tr>
<tr>
<td>• 0.4 mL Galacto-Light™ or Galacto-Light Plus™ substrate</td>
</tr>
<tr>
<td>• 40 μL Reaction Buffer Diluent</td>
</tr>
<tr>
<td>• 70 μL Light Emission Accelerator (Galacto-Light™ or Galacto-Light Plus™)</td>
</tr>
<tr>
<td>• 70 μL Lysis Solution</td>
</tr>
</tbody>
</table>

| **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 Galacto-Light™ or Galacto-Light Plus™ substrate |
| • 120 μL Reaction Buffer Diluent |
| • 210 μL Light Emission Accelerator (Galacto-Light™ or Galacto-Light Plus™) |
| • 210 μL 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 Galacto-Light™ or Galacto-Light Plus™ substrate |
| • 1 L Reaction Buffer Diluent |
| • 1.75 L Light Emission Accelerator-II (Galacto-Light Plus™) |
| • 1.75 L Lysis Solution |

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Learn more or order the products on this page at www.appliedbiosystems.com
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].
**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.

<table>
<thead>
<tr>
<th>ASSAYS PER KIT</th>
<th>BUFFER 1</th>
<th>BUFFER 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1035</td>
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<tr>
<td>T1033</td>
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<td>T1036</td>
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**APPLIED BIOSYSTEMS ORDER INFO**

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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.)


Learn more or order the products on this page at www.appliedbiosystems.com
Luciferase 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.

Product Configuration

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<thead>
<tr>
<th>Luciferase Assay System Standard Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capacity:</strong> 200 assays</td>
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<tr>
<td><strong>Contents:</strong></td>
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<tr>
<td>• 20 mL of Luciferase Assay System Substrate A*</td>
</tr>
<tr>
<td>• 20 mL of Luciferase Assay System Substrate B*</td>
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<td>• 70 mL of Luciferase Assay System Lysis Solution</td>
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<table>
<thead>
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<tr>
<td><strong>Contents:</strong></td>
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<td>• 3 x 20 mL of Luciferase Assay System Substrate B*</td>
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<td><strong>Contents:</strong></td>
</tr>
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</tr>
<tr>
<td>• 5 x 100 mL of Luciferase Assay System Substrate B*</td>
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<td>• 1.75 L of Luciferase Assay System Lysis Solution</td>
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*Supplied lyophilized

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

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

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

**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 65ºC, 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.**
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-placentale 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
  - 675 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

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

Immunodetection Products

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Immunodetection Products

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].
Additional Applications

Whole-cell ELISA
Immunodetection 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 and Sapphire-II™ enhancer for chemiluminescent immunodetection, particularly for whole cell ELISA applications [12] that may exhibit high levels of cellular alkaline phosphatase.

Protein Detection Applications
Antiphosphopeptide 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.

REFERENCES
Immunodetection Products

**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 microtiter plate; 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. The 96-well version can 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 Gs- or Gi-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.**

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

Learn more or order the products on this page at www.appliedbiosystems.com
## 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.

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### REFERENCES

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


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**Immunodetection Products**

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**cAMP-Screen® System, continued**

![Dynamic Range of cAMP-Screen® Assay](image-url)
Immunodetection Products

**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-precoated 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.

The 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 either in 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 Immunooassay 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 Immunooassay 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 assay 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.**
**Product Configurations**

The 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>T1505</th>
<th>T1506</th>
<th>T1507</th>
<th>T1508</th>
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<td>Plate Format</td>
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<td>96-well</td>
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<tr>
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<td>768</td>
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<td>2 L</td>
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<td>cAMP Standard</td>
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<td>5 mL</td>
<td>2 x 5 mL</td>
<td>100 mL</td>
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<tr>
<td>Anti-cAMP Antibody</td>
<td>14 mL</td>
<td>20 mL</td>
<td>2 x 35 mL</td>
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<tr>
<td>cAMP-AP Conjugate</td>
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<td>250 mL</td>
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<td>Conjugate Dilution Buffer</td>
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<tr>
<td>Wash Buffer</td>
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<td>1 L (1X)</td>
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<tr>
<td>CSPD®/Sapphire-II™ RTU</td>
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**APPLIED BIOSYSTEMS ORDER INFO**

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<tr>
<td>768 assays</td>
<td>T1506</td>
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<td>960 assays</td>
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<td>1 L</td>
<td>T2356</td>
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</table>

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**REFERENCES**


**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-NMC 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.
Immunodetection Products

ELISA-Light™ System

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**

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<td>100 mL total</td>
<td>T1023</td>
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<td>standard size</td>
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<td>standard size</td>
<td>T1025</td>
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<tr>
<td>standard size</td>
<td>T1026</td>
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</table>

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.**

Light is Produced Proportional to the Amount of Antigen

Learn more or order the products on this page at www.appliedbiosystems.com
Introduction to Chemiluminescent Membrane-based Immunodetection

Chemiluminescent Immunodetection
Western blotting is a widely used technique for the immunodetection of 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 platform.

Advantages of Chemiluminescence
Western-Light™ and Western-Star™ chemiluminescent Western blotting systems offer highly sensitive, rapid, nonisotopic protein detection. 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 lumino 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].

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.

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

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

Panel A. Nitrocellulose

Panel B. PVDF

(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 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.
**Immunodetection Products**

**Western-Star™ System**

**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].

![Western-Star™ vs. Enhanced Luminol Detection](image)

**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 antibody, 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.

![Western-Star™ System](image)

**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

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<tr>
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</tr>
<tr>
<td>with Goat Anti-Rabbit IgG AP Conjugate</td>
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<tr>
<td>Western-Star™ System Accessories</td>
<td>(available separately, see page 44)</td>
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<td>Goat Anti-Rabbit IgG AP Conjugate</td>
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<tr>
<td>Goat Anti-Mouse IgG+IgM AP Conjugate</td>
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<tr>
<td>I-Block™ Blocking Reagent</td>
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<td>Tropifluor™ PVDF Membrane (15 cm x 15 cm)—5 Membranes/Pack</td>
<td>1 pack</td>
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<tr>
<td>Development Folders (14 cm x 19 cm)—30 Folders/Pack</td>
<td>1 pack</td>
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REFERENCES

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

Western-Light™ System

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-picomole 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 (Avidx-AP™, see Reagents and Accessories section, page 44).

Product Configuration

<table>
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<th>Western-Light™ System Standard Size</th>
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<tr>
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</tr>
<tr>
<td>with Goat Anti-Rabbit IgG AP Conjugate</td>
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<td>T1047</td>
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</table>

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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**Immunodetection Products**

**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.

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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-picomole 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**
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**Product Configuration**

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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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**Immunodetection with Western-Light™ System.**

Protein transferred to membrane

Primary antibody and secondary antibody-alkaline phosphatase conjugate bound to protein

Alkaline phosphatase activates CSPD® substrates, producing light

Film image
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.

4

Nucleic Acid Membrane-Based Detection Products

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Southern-Star™ System .............................................. 41
Southern-Light™ System .............................................. 42
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.

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 [29]
- 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].
Nucleic Acid Membrane-Based Detection Products

Introduction, continued

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<th>Detection Scheme</th>
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<th>Special Features</th>
</tr>
</thead>
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<td>Most sensitive system for biotin detection</td>
<td>CDP®</td>
<td>StripFast AP Conjugate (Avidin/αAP™)</td>
<td>1 min–2 hr</td>
<td>Compared to 32P, 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</td>
</tr>
<tr>
<td>Southern-Light™ for detection of biotin-labeled probes</td>
<td>Established system with good sensitivity</td>
<td>CSPD®</td>
<td>StripFast AP Conjugate (Avidin/αAP™)</td>
<td>5 min–10 hr</td>
<td>Original system for chemiluminescent Southern blotting. Applications include Southern and Northern blotting, colony/plaque screening, and direct detection of AP-oligo probes.</td>
</tr>
</tbody>
</table>

Figure 54. Selection Guide for Southern/Northern Blot Detection Systems. Applied Biosystems offers several Southern blotting systems. High sensitivity in nonradioactive 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 sensitivity, 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 Biosystems website.)

4. Bronstein, I, JC Voyta, KJ Morrison, CS Martin, T Jaffe, RS Oosterhuis, B Edwards and LJ Kricka (1990). Rapid and sensitive detection of nucleic acids 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 sensitivity, is recommended to new users for all applications.
**Southern-Start™ System**

**Nucleic acid detection system for biotin-labeled probes**

**Description**

Southern-Start™ 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-Start detection of biotin-labeled nucleic acid probes incorporates CDP-Star® substrate and Avidx-AP™ streptavidin-alkaline phosphatase conjugate. Southern-Start 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-Start 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-Start system detection ideal for chemiluminescent phosphor screen and CCD camera imaging systems.

**Applications**

Southern-Start 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.

**Product Configuration**

**Southern-Start™ 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)

**Southern-Start™ 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)

**Southern-Start™ 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 1 roll T2232

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

**Figure 55. Southern-Start™ Nucleic Acid Detection with CDP-Star® vs. CSPD® Substrates.**

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

Learn more or order the products on this page at www.appliedbiosystems.com
Nucleic Acid Membrane-Based Detection Products

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)

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)

APPPLIED 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

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

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 MgCl₂.

**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/cm²) 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 mechan-