

# Tropix Chemiluminescent Kits and Reagents For Cell Biology Applications

## Introduction to Chemiluminescence

Chemiluminescence is the conversion of chemical energy to light energy. Tropix 1,2-dioxetane chemiluminescent substrates enable extremely sensitive detection of biomolecules by producing light that is detected with film or instrumentation. These reagents can be used in immunoassays, enzyme assays, reporter gene assays, membrane-based protein detection, and nucleic acid detection on membranes or in tube or microplate assay formats. Chemiluminescent detection offers substantial benefits that colorimetric, fluorescent, or isotopic detection cannot match.

Presented here are the most widely used Tropix chemiluminescent products to aid your research.

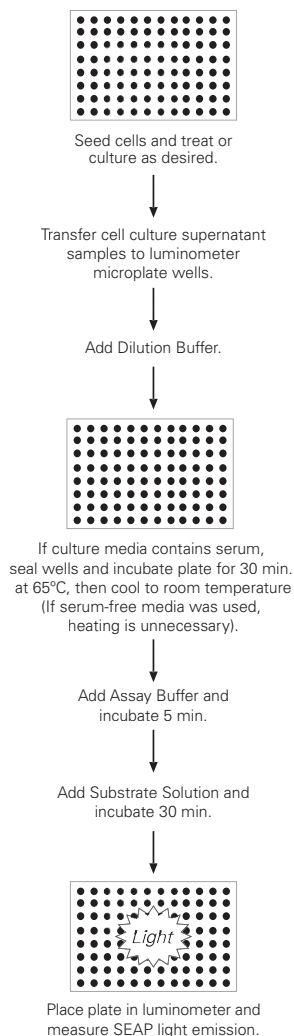
Reporter Assay System	Reporter Enzyme(s)	Key Attributes	Substrate	Duration of Light Emission	Reagent Injection	Special Features
<b>Gal-Screen®</b>	β-Galactosidase	Homogeneous assay does not require removal of culture medium.	Galacton-Star®	60-90 min (+)	Not required	Designed for assays on cells cultured in luminometer plates for high-throughput screening. Choice of reaction buffers for lysis of mammalian or yeast and mammalian cells.
<b>Galacto-Star™</b>	β-Galactosidase	Single step addition of substrate and enhancer	Galacton-Star®	1 hr (+)	Not required	Useful for high throughput applications. Choice of lysis buffer for yeast and mammalian model systems. Recommended for all new users.
<b>Galacto-Light Plus™</b>	β-Galactosidase	Extended light emission and better sensitivity than Galacto-Light™	Galacton-Plus®	30-60 min	Recommended	Established system with high sensitivity.
<b>Luc-Screen®</b>	Firefly Luciferase	Extended-glow homogeneous assay does not require removal of culture medium	Luciferin	90 min (+)	Not required	Designed for assays on cells cultured in luminometer plates for high-throughput screening.
<b>Dual-Light®</b>	Firefly Luciferase, β-Galactosidase	Detection of luciferase and β-galactosidase from same cell extract	Luciferin Galacton-Plus®	5 min/30 min	Required	Two assays from one cell extract enables better precision for normalizing transfection efficiency.
<b>Phospha-Light™</b>	Secreted placental alkaline phosphatase	Secreted reporter enzyme assayed in sample of culture medium	CSPD®	1-2 hr (+)	Not required	Cells remain viable; useful for stable transfectants, time course studies, etc. SEAP reporter enzyme can be measured from serum in in vivo animal studies

Figure 1. Selection Guide for Reporter Gene Assay Systems.

## Phospha-Light™ Assay System

The Phospha-Light™ Assay System is a chemiluminescent reporter gene assay for the sensitive detection of the secreted placental alkaline phosphatase (SEAP) reporter gene. The system, which requires no cell lysis, incorporates the CSPD® high performance alkaline phosphatase substrate, Emerald™ Luminescence Enhancer and a unique buffer designed for specific inhibition of endogenous non-placental alkaline phosphatase (PLAP) activity.

- Detects 10 fg or less SEAP
- Cells remain intact; can monitor cell populations over time
- Glow-light emission does not require luminometers with automatic injectors
- Wide linear range enables accurate intra-assay comparison; no need for multiple sample dilutions
- SEAP can be measured from serum in in vivo animal studies

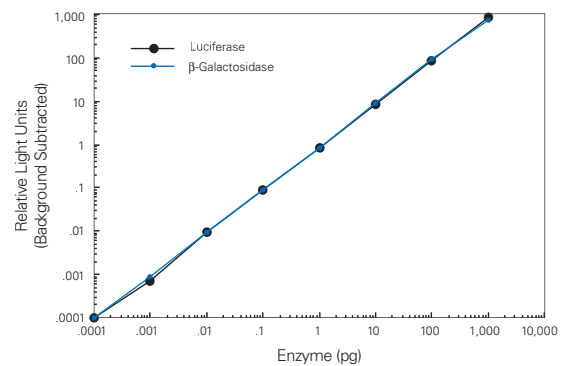


**Figure 2.** Secreted Alkaline Phosphatase Reporter Gene Assay with Phospha-Light™ System.

## Dual-Light® Assay System

The Dual-Light luminescent reporter gene assay is designed for the rapid and sensitive detection of firefly luciferase and  $\beta$ -galactosidase in the same sample. The use of two reporter genes, one as an experimental reporter and the other as a constitutively expressively transfection control reporter, is widely used for accurate quantitation of activity from experimental reporter constructs.

- Assay complete in less than 1 hour; saves time and money
- Ideal for transfection normalization
- Low detection limit of reporter proteins (fg level of sensitivity)
- Wide dynamic range (fg to ng)
- Compatible with the Ambion pMIR-REPORT™ miRNA Expression Reporter Vector System



**Figure 3.** Detection of Firefly Luciferase and  $\beta$ -Galactosidase with Dual-Light® System.

## Galacto-Star® Assay System

The Galacto-Star® chemiluminescent assay provides sensitive, detection of as little as 10 fg of  $\beta$ -galactosidase (20,000 molecules) in cell lysates. This high sensitivity makes the system ideal for the detection of weak expression and for transfection normalization with other sensitive reporter gene assays. The Galacto-Star® System uses a one-step assay, following lysis preparation, which simplifies detection.

- Most sensitive reporter gene assay for  $\beta$ -galactosidase
- Requires no automatic reagent injection
- Light emission reaches maximum in 60–90 minutes and remains constant for 1 hour
- Wide application for assays that use  $\beta$ -galactosidase reporter as a read-out for gene expression
- Detection of  $\beta$ -galactosidase activity in mammalian or yeast cell lysates

## Gal-Screen® Assay System

The Gal-Screen® Assay System combines direct cell lysis with rapid ultra-sensitive chemiluminescent detection of the  $\beta$ -galactosidase reporter enzyme. The Gal-Screen system is ideally suited for screening applications that require assay automation. It provides greater sensitivity than colorimetric or fluorescent assays, with a lower limit of detection of 1 pg in the presence of culture medium. The system is ideally suited for high-throughput screening applications.

- Adaptable for use in 96-, 384-, or 1536-well plates
- Saves time and money: single-step cell lysis and reagent addition step; no sample prep
- No special culture media or media removal required
- Superior dynamic range: pg to ng level of detection

## Luc-Screen® Assay System

The Luc-Screen® Assay System with extended-glow light emission is designed for sensitive detection of the firefly luciferase reporter enzyme. Luciferase is an ideal reporter because of its sensitive detection capability and the absence of endogenous luciferase activity in mammalian cells. The system produces a stable glow signal of at least 90 minutes and is compatible with media containing phenol red. The assay is especially effective for high-throughput screening assays.

- 10-minute in-well lysis
- Requires no special culture media or media removal
- Long-lived light emission (4–5 hours) provides measurement flexibility after reagent addition
- Wide dynamic range: 50 fg–100 ng

## Immunodetection Products

### cAMP-Screen® Immunoassay System

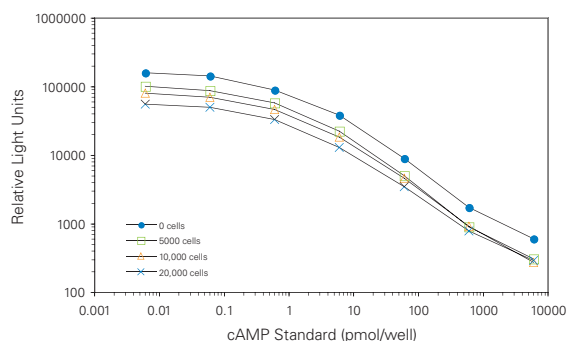
The cAMP-Screen® Immunoassay System enables ultrasensitive determination of cyclic AMP (cAMP) levels in cell lysates. The assay is formatted with maximal flexibility to permit either manual assay or automated high-throughput screening. The cAMP-Screen assay uses the highly sensitive chemiluminescent alkaline phosphatase (AP) substrate CSPD® with the Sapphire-II™ Luminescence Enhancer. This system has the broadest dynamic range (4–5 logs) of all available products, produces no false positives, and requires no sample dilution.

- CVs as low as 3% intra-assay precision
- Detect as few as 60 fmol of cAMP in a 96-well system
- 96- or 384-well format

### cAMP-Screen Direct® Cyclic AMP Immunoassay System

The cAMP-Screen Direct® Cyclic AMP Immunoassay System enables ultrasensitive determination of cyclic AMP (cAMP) levels in cell lysates. Formatted with maximal flexibility to permit either manual assay or automated high-throughput screening, the cAMP-Screen assay uses the highly sensitive chemiluminescent alkaline phosphatase (AP) substrate CSPD® and Sapphire-II™ Luminescence Enhancer. The ready-to-use enhancer generates sustained glow-light emission that is measured 30 minutes after addition.

- Provides the highest sensitivity of any commercially available cAMP assay
- Detects as few as 60 fmol of cAMP
- Improved precision with one less transfer step
- Saves time and money by eliminating a cell culture plate and sample transfer



**Figure 4.** Sensitivity of the cAMP-Screen Direct® System. cAMP assay performed with the cAMP-Screen Direct system. cAMP standards were used in a cAMP-Screen Direct assay plate following growth of different densities of HEK293 cells in assay wells. Detection sensitivity of exogenously added cAMP is unchanged following growth of cells in assay plate. Signal intensity differences result from basal cellular levels of cAMP. Signal was measured with the TR717™ microplate luminometer.

### ELISA-Light™ Immunoassay System

The ELISA-Light™ Immunoassay System is designed for the sensitive immunodetection of proteins, nucleic acids, and other molecules using an alkaline phosphatase (AP) labeled detection antibody or probe. More sensitive than colorimetric or isotopic methods, this system has a broad dynamic range and is compatible with multiple immunoassay formats.

- Optimized, ready-to-use reagents for developing customized chemiluminescent ELISAs
- AP labels with 1,2-dioxetane substrates—the standard for high sensitivity ELISAs
- Rapid antigen detection in enzyme immunoassays
- Available with multiple substrates and related luminescent enhancers

## ELISA-Light™ System Standard Size:

Capacity: 500-1,300 assays

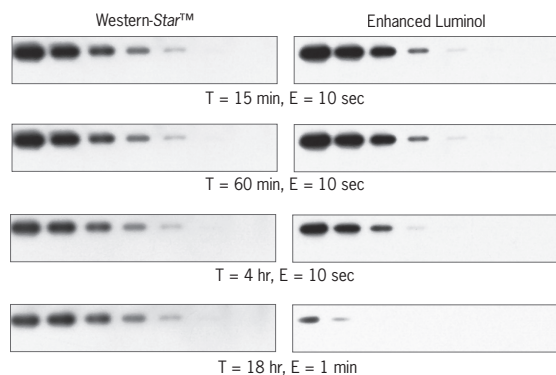
Contents:

- 100 mL of Ready-to-Use chemiluminescent substrate with enhancer
- 100 mL of 10X Assay Buffer
- 7.5 g of I-Block™ blocking reagent

## Western-Star™ System

The Western-Star™ Immunodetection System is a highly sensitive chemiluminescent immunodetection system for measuring protein knockdown in RNAi experiments. The system is fast. It can be used with a variety of imaging systems. Western-Star generates a high-intensity chemiluminescent signal that persists from hours to days, depending on the membrane. It is designed for chemiluminescent detection of proteins in western blotting applications with AP-labeled secondary antibodies.

- Signal detection by film or CCD imaging systems
- Highest sensitivity immunodetection
- Superior band resolution
- Compatible with PVDF, nylon, and nitrocellulose membranes
- Long-lived light emission enables both immediate and next-day imaging exposures.

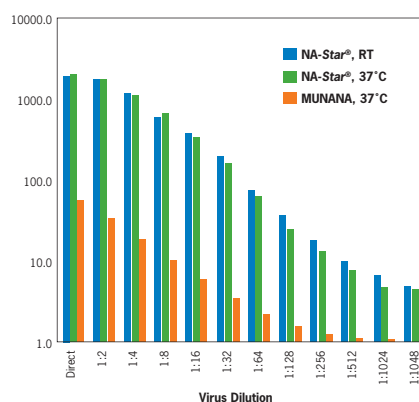


**Figure 5.** Western-Star™ System vs. Enhanced Luminol Detection. Human brain extract was electrophoretically separated and transferred to PVDF. Blots were incubated with a monoclonal anti-actin, followed by Western-Star™ detection or with a horseradish peroxidase (HRP)-conjugated secondary antibody,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

## NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit

The NA-Star® chemiluminescent substrate enables sensitive detection of neuraminidase (NA) activity. The kit includes reagents to quantitate such activity as well as NA inhibitor resistance in avian, equine, human (types A and B), and porcine influenza viruses. It includes a fast (<1.5 hr) and easy protocol and convenient 96-well plate format.

- Ideal for monitoring influenza virus neuraminidase inhibitor resistance and high-throughput inhibitor compound screening
- Highest sensitivity for NA activity detection—up to 50-fold higher than MUNANA assays
- High dynamic range allows quantitation across a broad range of virus concentrations and varying levels of NA activity



**Figure 6.** Sensitivity Comparison of Chemiluminescent Assay Detection with NA-Star® Substrate to Fluorescent Assay Detection with MUNANA Substrate. Dilutions of Influenza Type B (ATCC VR-1535) virus culture supernatant (cultured on MDCK cells) were assayed at different temperatures and Signal/Noise (S/N) ratio calculated using uninfected MDCK cell supernatant (Noise). The lower limit of detection (S/N = 2) is at least 30-fold lower with the chemiluminescent NA-Star assay, and S/N is approx. 50-fold higher with NA-Star assay. The dynamic range of detection with the NA-Star assay with virus samples is three orders of magnitude.

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**ORDERING INFORMATION**

<b>Description</b>	<b>Size</b>	<b>P/N</b>	<b>Price</b>
<b>Reporter Gene Assays</b>			
Dual-Light® Assay System	Standard size	T1003	\$260
Gal-Screen® System for Mammalian Cells	200 assays	T1029	\$150
	1,000 assays	T1027	\$475
	10,000 assays	T1028	\$3,000
Gal-Screen® System for Yeast or Mammalian Cells	200 assays	T1032	\$150
	1,000 assays	T1030	\$475
	10,000 assays	T1031	\$3,000
Galacto-Star® Mammalian Cell Reporter Gene Assay System	Standard size	T1012	\$175
	Large size	T1014	\$400
	Screening size	T1013	\$3,000
Galacto-Star® Yeast Cell Reporter Gene Assay System	Standard size	T1019	\$225
	Large size	T1021	\$500
	Screening size	T1020	\$3,100
Luc-Screen® Extended-Glow Firefly Luciferase Assay System	200 assays	T1035	\$150
	1,000 assays	T1033	\$550
	5,000 assays	T1036	\$1,600
	10,000 assays	T1034	\$3,000
Phospha-Light™ Reporter Gene Assay System	Standard size	T1015	\$200
	Large size	T1017	\$400
	Screening size	T1016	\$3,000

*continued on next page*

## ORDERING INFORMATION

Description	Size	P/N	Price
<b>Immunodetection Products</b>			
cAMP-Screen® 96-Well Immunoassay System	192 assays	T1500	\$550
	768 assays	T1502	\$2,500
	960 assays	T1501	\$1,800
	19,200 assays	T1504	\$14,500
cAMP-Screen Direct® 96-Well Immunoassay System	192 assays	T1505	\$575
	768 assays	T1506	\$1,900
	960 assays	T1507	\$2,600
	19,200 assays	T1508	\$15,000
ELISA-Light™ Immunoassay System	Sampler size	T1022	\$400
100 mL CSPD® 0.4 mM ready to use with Sapphire-II™	Standard size	T1023	\$325
100 mL CSPD® 0.4 mM ready to use with Emerald-II™	Standard size	T1024	\$325
100 mL CDP-Star® 0.4 mM ready to use with Sapphire-II™	Standard size	T1025	\$375
100 mL CDP-Star® 0.4 mM ready to use with Emerald-II™	Standard size	T1026	\$375
<b>Other Products</b>			
NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit	960 assays	4374422	\$960
NA-Star® Influenza Neuraminidase Inhibitor Resistance Reagent Set	960 assay wells	4374348	\$920
NA-Star® Detection Microplates	10 × 96-wells	4374349	\$80
Western-Star™ System with Goat Anti-Mouse IgG+IgM AP Conjugate	Standard size	T1046	\$325
Western-Star™ System with Goat Anti-Rabbit IgG+IgM AP Conjugate	Standard size	T1048	\$325

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