BIOSYNTH®
Carbosynth

X-Shining™ Thermostable Luciferase

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Biosynth Carbosynth's modified thermostable X-Shining™ Luciferase is perfectly suitable for any luciferin-luciferase-based assay using D-luciferin (dLuc) or synthetic pro-luciferins (caged luciferins). Examples of its typical applications include ATP tests in hygiene monitoring, ATP tests in drug screenings and bacteria identification in microbial assays. Our new luciferase has been optimised by genetic engineering for strongly increased thermostability and storage stability. X-Shining™ Luciferase is supplied as aqueous solution with glycerol, and it may be kept for months at room temperature without significant loss of function. In temperature stress tests, the enzyme survives temperatures of 60°C for over an hour, whereas a normal luciferase from firefly is inactivated after only a few minutes. This makes it user-friendly and eliminates some of the main disadvantages and limitations of the commonly used wild type luciferase. By combining genetic engineering, protein over-expression and purification techniques, we can produce a more than 90% pure luciferase enzyme that is more cost effective than even wild-type recombinant luciferase from Photinus pyralis.

X-Shining™ Luciferase is the most robust, thermostable luciferase on the market, at the most competitive price.

CAS Number	61970-00-1
Code	BX174908, L-8093
Product	X-Shining™ Luciferase
Description	Thermostable modified recombinant luciferase from the North-American firefly *Photuris pennsylvanica*. Supplied as 10 mg/mL aqueous solution with glycerol.

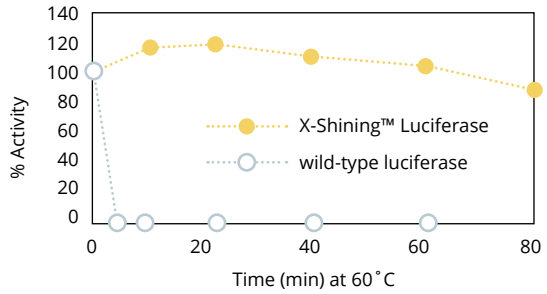
Quantity	Price ($)
100 µL (1 mg)	115.00
250 µL (2.5 mg)	275.00
500 µL (5 mg)	500.00
1 mL (10 mg)	850.00

Introduction

Bioluminescence offers huge advantages over conventional chromogenic or fluorescent methods for sensitive detection systems, as it measures light signals against a completely dark background. It is like observing stars in front of a perfectly black night sky. The fantastic sensitivity and the relatively low instrumentation requirements have ensured that bio- and chemiluminescence methods have replaced other measurement methods in many areas in recent years.

A Luciferase-based reporter gene assay in molecular genetics consists in the transfection of a vector that contains the promoter and the sequence for the expression of the Luciferase enzyme. The promoter is activated in the presence of a specific transcription factor, which causes the production of Luciferase which undergoes the typical light emission reaction. Reporter gene assays are widely employed to study gene expression, post-transcriptional modifications, protein/protein interactions, signal transduction pathways as a tool in gene therapy and high throughput screening for drug discovery.

Bioluminescence-based methods have also great potential for tests in hygiene monitoring, microbiological food and drinking water control, as well as clinical microbiology. However, the use of luciferase enzyme for in vitro assays has been limited by the availability of a cost-efficient stable Luciferase enzyme. With the new X-Shining™ Luciferase, an inexpensive stable luciferase is now commercially available.



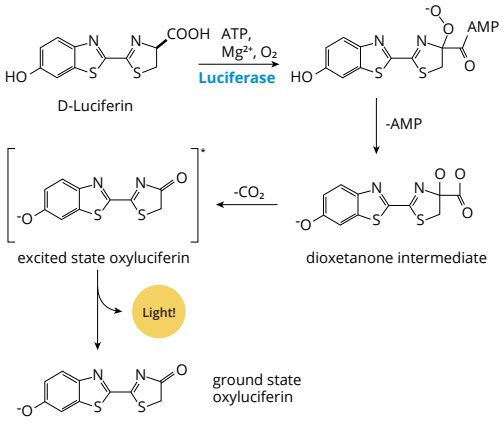
Comparison of the heat stability of X-Shining™ Luciferase and wild-type recombinant luciferase (from ***Photinus pyralis***) after storage at 60°C.

X-Shining™ Luciferase fully retained its activity after 60 min at 60°C, while standard luciferase was completely inactivated after just 10 min.

Additionally with X-Shining™, the luminescence signal stability is noticeably increased in comparison to recombinant wild-type luciferase from firefly (data not shown).

Luciferin/Luciferase Mechanism of Light Emission

In order to observe light emission, the oxidative transformation of Luciferin into an excited state intermediate by Luciferase needs to take place. The reaction occurs in the presence of oxygen, ATP and Mg^{2+} co-factors. The main pathway of the bioluminescent reaction begins with the formation of Luciferyl adenylate by reaction of Luciferin with ATP. Oxidation of Luciferyl adenylate occurs through a single electron transfer mechanism, and gives an intermediate peroxide which further converts into dioxetanone. Decomposition of the dioxetanone intermediate occurs with concomitant release of CO_2 and gives the excited state oxyluciferin, which returns to its ground state by emitting light. Natural D-Luciferin reacts with luciferase emitting light at 557 nm (green-yellow).

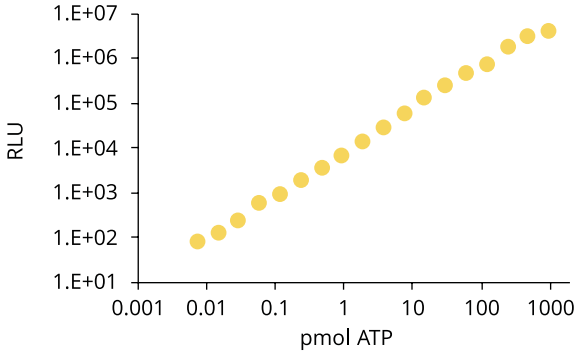


Application Examples for the Thermostable X-Shining™ Luciferase

Example 1: ATP Assays

Adenosine triphosphate (ATP) is present in all living cells and plays a central role in the energy balance. The intracellular concentration of ATP is tightly regulated and is maintained at a similar level in all cells. When a cell dies, the ATP is completely degraded; ATP levels therefore reflect the presence of any living cell. The bioluminescence-based assays are extremely sensitive; a standard luminometer can detect as little as 0.1 picomole of ATP.

The sensitivity has led to numerous applications as detecting low-level bacterial contamination in samples such as blood, urine and milk. ATP production, measured by the luciferin-luciferase assay has also been used successfully to study the effects of antibiotics on bacterial populations.

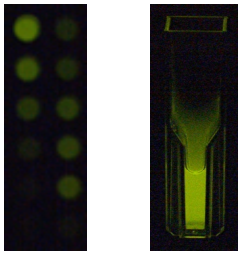


ATP Quantification with X-Shining™ Luciferase

X-Shining™ Luciferase in combination with D-Luciferin shows an excellent signal linearity over a wide ATP concentration range, from 8 fmol to 1 nmol in 96-well plate assays with 0.1 mL liquid volume.

The **Luciferin-Luciferase** system is an extremely sensitive and accurate tool for ATP concentration determination.

Example 2: Detection of Enzyme Activities Using Luciferase with Pro-Luciferins (Caged Luciferins)



Enzyme activity detection using Luciferin-Luciferase in microplate (left) or tube test (right) format.

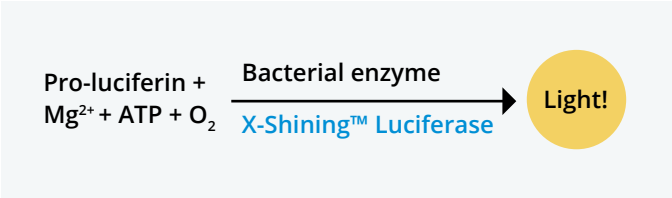
Luciferase bioluminescence can also be used for ultra-sensitive detection of a broad range of enzymatic activities, using D-luciferin or aminoluciferin coupled with enzyme labile groups as substrates. Luciferin-based substrates have been referred to as "caged luciferins" or "pro-luciferins" in the literature. Pro-lucferins can be used for example for the detection of specific groups of bacteria, for reporter gene assays in animal cells or plant biology.

Coupled enzyme assays can be performed in the same reaction including the sample, pro-luciferin substrate and the luciferase reaction. To precisely determine the enzyme activity in a sample, an enzyme concentration series is measured under identical reaction conditions. The luminescence signal of a sample is then compared with the enzyme concentration series.

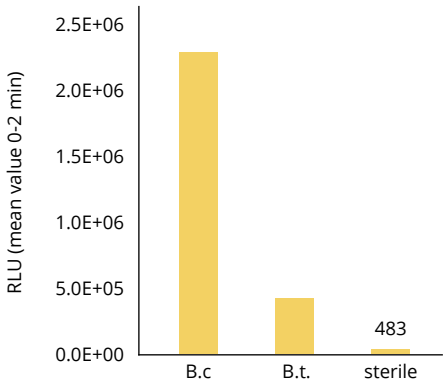
Due to the availability of a thermostable enzyme, the number of possible bioluminescence applications increases enormously.

Example 3: Bacteria Detection with Pro-Luciferins

The introduction of pro-luciferins into microbial analytics has opened the way for new bioluminescent-based bacteria detection methods. In this setup, bacteria cultures are grown to relatively low bacteria concentration and subsequently supplied with the bio-luminescent substrate, i.e. the pro-luciferin. The bacteria-specific enzyme cleaves off the enzyme-labile group from the substrate, leading to the release of D-luciferin. Then, in the presence of ATP, magnesium ions and oxygen, the reaction of D-luciferin with luciferase leads to light emission.



Luciferase can detect luciferin even at very low concentrations, and therefore enables the lowest detection limits for vital bacteria. For example in the identification of bacteria positive for phosphatidyl-choline specific phospholipase C (PC-PLC), such as strains of Pseudomonas aeruginosa and Bacillus cereus.



Light emission of bacterial cultures and sterile control with D-Luciferin-choline-phosphate, ATP and luciferase. Recorded with a plate reader at room temperature. The sample from the ***B. cereus*** culture (B.c.) exhibited strong luminescence, while RLU values were significantly lower for samples from the PC-PLC negative ***B. thuringiensis*** culture (B.t.) and the sterile control.

Thermostability of the Enzyme — Advantages of practical use:

Due to the excellent stability of our enzyme filter-sterilised solutions of X Shining™ Luciferase in assay buffer can be kept for at least seven months at room temperature, with an even longer storage time if refrigerated.

Enlightening! – Biosynth Carbosynth luciferin in combination with X-Shining™ Luciferase.

Related Products, Examples

Luciferins

CAS Number	Code	Product	Quantity	Price ($)
115144-35-9	L-8221	D-Luciferin Firefly, potassium salt - Endotoxin-free	0.25 g 0.5 g	188.00 258.00
115144-35-9	L-8230	D-Luciferin Firefly, potassium salt - white	0.05 g 0.1 g 0.5 g	120.00 192.00 460.80
103404-75-7	L-8241	D-Luciferin Firefly, sodium salt monohydrate - Endotoxin-free	0.25 g 0.5 g	188.00 258.00
2591-17-5	FL08607	D-Luciferin free acid	25 mg 250 mg	23.25 147.50
115144-35-9	FL08608	D-Luciferin potassium salt	50 mg 500 mg	50.00 215.00
103404-75-7	FL08609	D-Luciferin sodium salt	100 mg 500 mg	65.00 225.00

Proluciferins and ATP

CAS Number	Code	Product	Quantity	Price ($)
131474-38-9	EL28834	D-Luciferin-6-O b-D-galactopyranoside	1 mg 10 mg	57.80 378.00
	EL08610	D-Luciferin-6-O b-D-glucopyranoside	5 mg 50 mg	108.00 730.00
	L-8122	D-Luciferin-beta-D-glucuronide, dipotassium salt	5 mg 50 mg	90.00 607.50
	L-8281	Luc-Salmonella (D-Luciferin caprylate)	5 mg 50 mg	120.00 810.00
	L-8120	D-Luciferin-myo-inositol-1-phosphate, sodium salt	5 mg 50 mg	157.50 1057.50
	L-8275	Luc-B.cereus Na (D-Luciferin-6-O-cholin phosphate sodium)	5 mg 50 mg	175.00 1175.00
34369-07-8	NA00135	Adenosine 5'-triphosphate disodium salt hydrate	100 g 500 g	65.00 225.00

Prices subject to changes. Visit our website or contact our sales team at ***sales@biosynth-carbosynth.com*** for updates.

Literature

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