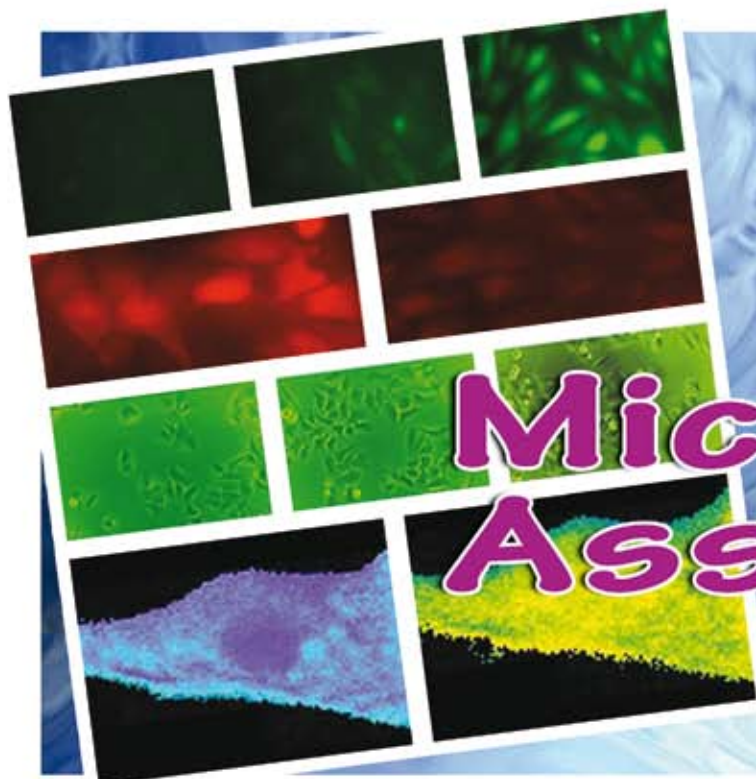


# Horizons Nouveaux Interchim Innovations

11-2008

211 bis avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - Tel 33 (0)4 70 03 88 55 - Fax 33 (0)4 70 03 82 60 - email [interchim@interchim.com](mailto:interchim@interchim.com) - [www.interchim.com](http://www.interchim.com)



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- Calcium Assays
- Viability & Cytotoxicity Assays
- Apoptosis Assays
- DNA, Proteins & Glucides Biochemistry Assays
- Immunological Assays
- Accessory tools



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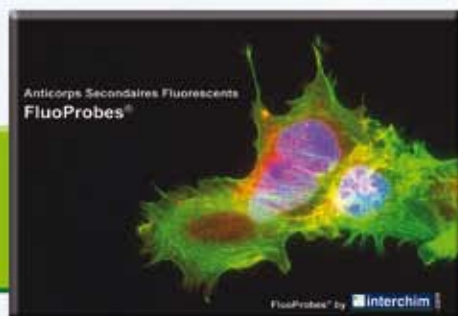
# Edito

This HN Microplates Assays presents a selection for reagents and kits to use in microplate readers. All the applications can be performed on the microplate instruments from our partner BERTHOLD TECHNOLOGIES.

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## Interchim and Berthold Technologies collaboration

Interchim, a provider of consumables for life sciences, and Berthold Technologies, a leader in microplate instrumentation technology, have entered into a collaboration agreement to offer complete instrumentation and reagent solutions.

BERTHOLD TECHNOLOGIES provides with the Mithras and TriStar extremely versatile multimode readers for all comprehensive technologies used in today's laboratory.

Additionally dedicated microplate readers for luminescence, fluorescence and absorbance can be offered for all common microplate formats. Petri dishes and Terasaki plates can be measured with respective adapters. Powerful software allows kinetics, scanning, repeated mode, dual ratio measurements etc.

For higher throughput the instruments can be run with the Stacker LB 931. Robot access enables integration into robotic HTS systems.



**Mithras LB 940**



**TriStar LB 941**

For instrument informations, please contact :

**Berthold France SAS**

Parc Technologique des Bruyères  
8, route des Bruyères  
78770 Thoiry  
France

Phone : (+33) 1 34 94 79 00

Fax : (+33) 1 34 94 79 01

E-mail : [France@Berthold.com](mailto:France@Berthold.com)





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# Firefly Luciferase



Interchim provides kits and stand-alone reagents to study the expression of Luciferases from different species. Beside the classical *Firefly* and *Renilla* luciferases, we also offer with the new *Gaussia* luciferases for improved signal intensity :

## Comparison of different species luciferases

Species	Luciferase	Size	Quantum Yield	Wavelength	ATP dependency	Substrate
<i>Photynus pyralis</i> (Firefly)	Fluc	550 aa	>88%	562 nm	YES	D-luciferin
<i>Renilla reniformis</i> (Sea pansy)	RLuc	311 aa	>6%	480 nm	NO	coelenterazine
<i>Gaussia princeps</i> (Copepod)	Gluc	185 aa	$1.6 \times 10^{16}$ Qps/mg	480 nm	NO	coelenterazine

Firefly luciferase is widely used as a reporter gene for studying gene regulation and function, and for pharmaceutical screening. It is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells or tissues. The Firefly luciferase is a 62 000 Dalton protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation by oxygen into oxyluciferin with emission of light centered on 562 nm (figure 1).

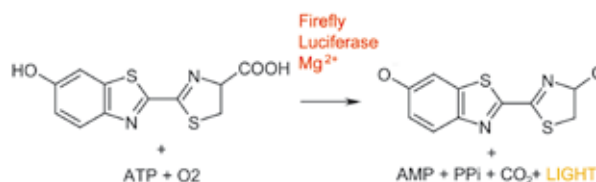


Figure 1 : Bioluminescent reaction catalyzed by Firefly luciferase.

However, the light production resulting from the reaction leads to formation of suicidal adenyloxyluciferin at the enzyme surface. It results in very short half-life of the light emission with a flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface. But the duration (10-15 min) is still too short for batch process screening.

Our luciferase assay kits provide a long lasting signal (steady glow) by preventing the formation of adenyloxyluciferin at the enzyme surface.

## Technical tip

### Microplate Readers

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with Berthold Technologies microplate readers.

#### \*Mithras LB940 MultiMode Reader.

Includes a variety of technologies with samples injectors and robot integration module.

- . Various formats (from Petri dishes to 1536 well plates)
- . Absorbance
- . Luminescence Flash & Glow
- . Fluorescence
- . top and bottom measurement
- . Polarisation (FP)
- . FRET
- . BRET
- . AlphaScreen™
- . TRF & HTRF® (Time Resolved Fluorescence & Time Resolved FRET)



#### \*Centro XS LB960 Luminometer

A robust, versatile and sensitive microplate luminometer (lowest crosstalk) exists also in a Clinical version (LB961).

#### \*Twinkle LB970 Fluorometer

Reading from above and below, from Petri dishes to 864 well microplates. Ideal for sensitive FRET assays

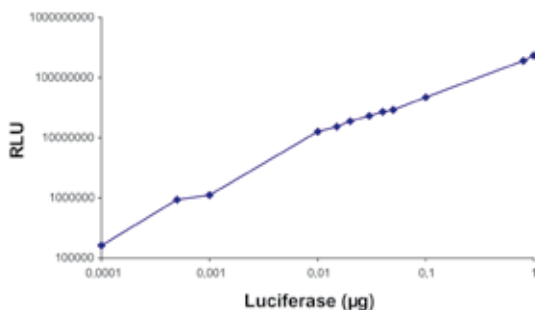
#### \*Apollo LB912 Absorbance Reader

96 wells microplate in 340-800 nm range, with 8-channels



## ■ Firefly Luciferase 1-Step Assay Kit, 2 h reading

- ▶ Linear range – Assay **linear over seven orders** of magnitude
- ▶ Limit of detection – less than **1 fg of luciferase** per sample
- ▶ **No disposal problems or hazards** are associated with the use of this luciferase assay kit
- ▶ Reproducibility – CV less than 5%



Sensitivity study on the Mithras luminometer from Berthold Technologies : Different quantities of Firefly Luciferase on the range of 0.0001-1 µg (0.005-50 µg/ml) have been assayed.

Luciferase 1-Step assay system is a homogeneous high sensitivity firefly luciferase reporter gene assay kit with a half-life of 2 hours for the quantification of firefly luciferase expression in mammalian cells. This kit is specially designed for batch processing systems using microplates such as 96-well plates. In addition, Interchim Luciferase 1-Step assay kit offers higher sensitivity and wider dynamic range for detecting luciferase activity within mammalian cells, consistent reproducibility and cost effectiveness along with the added convenience of a one step assay.

Description	P/N :	Qty
Firefly Luciferase 1-Step Assay Kit	<b>FP-BX0320</b>	100 ml (1000 tests in 96-well plate)
	<b>FP-BX0321</b>	100 ml (1000 tests in 96-well plate)

## ■ Firefly Luciferase Stable Assay Kit, 3-5 h reading

- ▶ **Simple** : single step assay
- ▶ **High sensitivity** : higher sensitivity than others steady substrates
- ▶ **Suitable for HTS** - batch processing

This kit is a homogeneous high sensitivity firefly luciferase reporter gene assay kit with a half-life of 3-5 hours for the quantification of firefly luciferase expression in mammalian cells. It is specifically designed for batch processing systems using high-density microplates such as 384- and 1536-well plates, in high throughput environments.

Description	P/N :	Qty
Firefly Luciferase Stable Assay Kit, 3-5 h reading	<b>FP-BU6870</b>	10 ml
	<b>FP-BU6871</b>	100 ml
	<b>FP-BU6872</b>	1000 ml

Kit contents (10/100/1000 ml) :

1 vial (2.5 /25 /250 mg) of D-Luciferin

1 bottle (10 /100/ 1000 mL) Firefly Assay Buffer

10 ml are sufficient for 100, 400 and 3,300 assays in 96-well, 384-well and 1536-well microplates.

## ■ Firefly Luciferase, recombinant, from *Photinus pyralis*

Luciferase can be used to detect trace amounts of ATP (signalling biological contamination). Less than or equal to one femtomole of ATP can be detected using 0.2 µg of luciferase.

Recombinant Firefly luciferase can also be used to prepare standard curve of reporter genes for the study of gene expression.

Description	P/N :	Qty
Firefly Luciferase, recombinant, from <i>Photinus pyralis</i>	<b>FP-D1826B</b>	1 mg

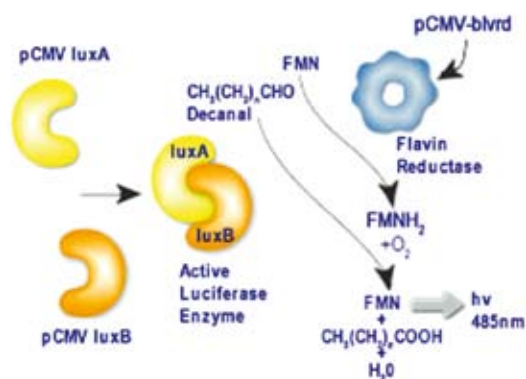
# Firefly Luciferase



## ■ Mammalian Expression Vectors : pCMVLuxA & pCMVLuxB

A single reporter to monitor expression of two cloned genes

The light-emitting reaction of the marine bioluminescent bacterium *Vibrio harveyi* is catalyzed by the bacterial luciferase enzyme which exists as an alpha-beta heterodimer encoded by the luxA and luxB genes with subunit molecular weights of 42K and 37K respectively. The enzyme catalyzes a reaction with FMNH<sub>2</sub>, oxygen and a long-chain aldehyde as substrates to yield visible light at 490 nm. A new luciferase marker gene detection system has been developed based upon this bacterial luciferase isolated from *Vibrio harveyi*. Sequences encoding the two luciferase subunits, luxA and luxB have been cloned into two separate vectors. These vectors also include a CMV promoter for expression in mammalian cells as well as an ampicillin resistance gene (100 ug/mL ampicillin resistance) for selection and amplification, the SV40 polyadenylation sequence and the SD/SA-RNA splice donor and acceptor sequence for maximum expression. In addition, the LuxA or LuxB gene can be excised using the flanking NotI sites to allow the insertion of other genes to be expressed under the same regulatory elements in mammalian cells. These systems are being developed to monitor regulation of expression for two independent vector constructs, upon the dual expression.



Description	P/N :	Qty
pCMVLuxA Mammalian LuxA Expression Vector	<b>D08130</b>	20 µg
pCMVLuxA Mammalian LuxB Expression Vector	<b>D08140</b>	20 µg

## ■ Firefly luciferase siRNA constructs : siFLuc

siFLuc are siRNA constructs designed to knock down Firefly luciferase.

In our control experiments, this siRNA can knock down Firefly luciferase activity by ~80%.

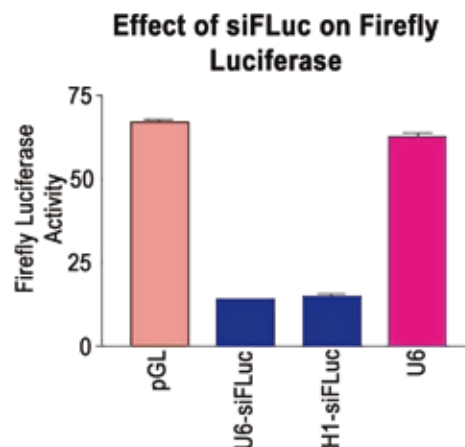
The target sequence matches pGL3-control, and is designed to silence Firefly luciferase expressed by a co-transfected pGL3-control vector.

Description	P/N :	Qty
pRNA-U6.1/Neo/siFLuc (positive control in mammalian transfection)	<b>BG9670</b>	10 µg

pRNA-U6.1/Neo/siFLuc is a siRNA expression vector designed for mammalian transfection. It uses U6 promoter for siRNA expression. This vector contains siRNA construct for firefly luciferase, and can be used as a positive control. It can also be used as a siRNA vector using BamH I and Hind III for siRNA insertion.

Please contact us for others promoters available :

- pGL : HEK293 cells transfected with pGL3-control (0.16 ug) and pRL-TK (0.16 ug)
- U6-siLuc : HEK293 cells transfected with pGL3-control (0.16 ug), pRL-TK (0.16 ug), and 1.6 ug of pRNA-U6.1/Neo/siLuc
- H1-siLuc : HEK293 cells transfected with pGL3-control (0.16 ug), pRL-TK (0.16 ug), and 1.6 ug of pRNA-H1.1/Neo/siLuc

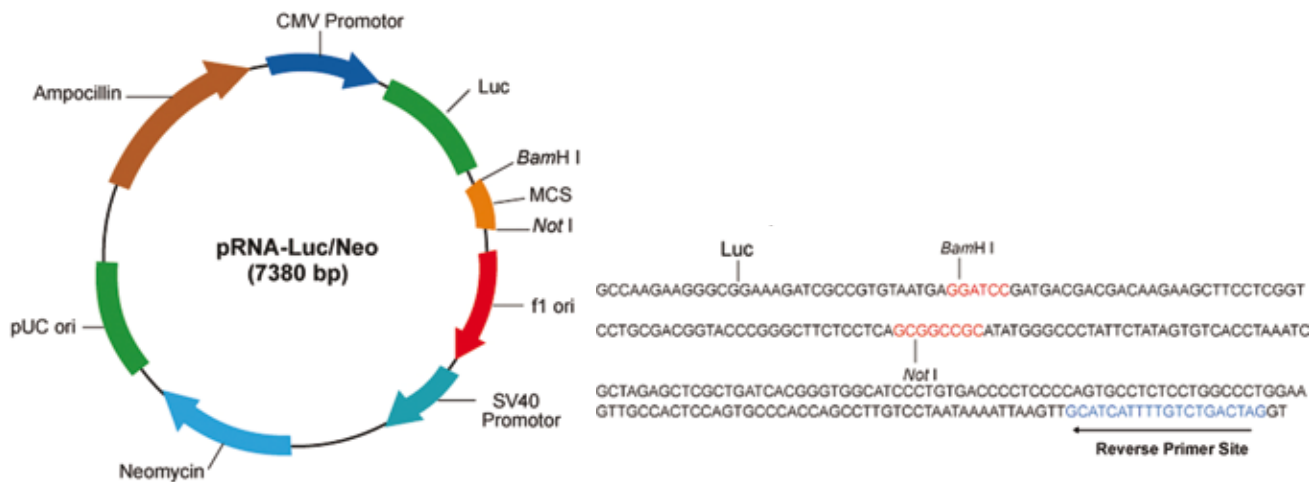


U6: HEK293 cells transfected with pGL3-control (0.16 ug) and pRL-TK (0.16 ug), and 1.6 ug of pRNA-U6.1/Neo empty vector.



## ■ pRNA Luc/Neo for monitoring transcriptional activity

The pRNA-Luc/Neo includes a modified coding region for firefly (*Photinus pyralis*) luciferase that has been optimized for monitoring transcriptional activity in transfected eukaryotic cells. The purpose of this reporter vector is to screen for efficient siRNA for the target gene using Luc activity as a reporter gene. The principle is that when a siRNA silence the target gene by degrading mRNA, the Luc will not be expressed either, because the mRNA for both the gene and Luc as a whole molecule is degraded. The assay of this genetic reporter is rapid, sensitive and quantitative.



Description	P/N :	Qty
pRNA-Luciferase-Neomycine	DT3120	10 µg
<b>Related product</b>		
β-Amyloid (1-40)	HT8360	0.5 mg
	HT8361	1 mg

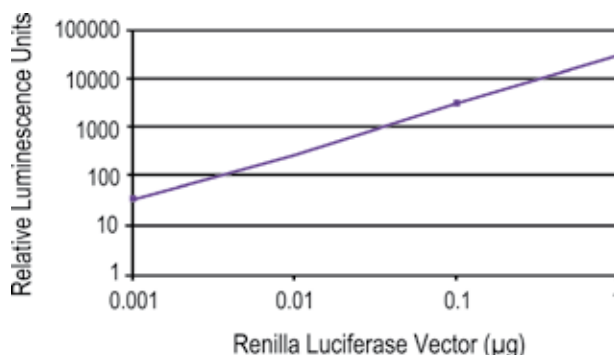


## ■ Renilla Luciferase Assay Kit

Reporter gene used as normalizing transfection control

- ▶ **Sensitivity and Linearity** : Linear correlation between luciferase gene expression and light output for transfection using 1 ng to 1 µg DNA of a Firefly luciferase reporter construct
- ▶ **Conveniently packaged** substrate sizes permitting you to run a variable number of assays

Renilla luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo. Recently, Renilla luciferase has been widely used in multiplex transcriptional reporter assays or as a normalizing transfection control for Firefly luciferase assay. Renilla luciferase, a monomeric 36 000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light. The enzyme does not require post-translational modification for its activity, and may function as a genetic reporter immediately following translation. native Coelenterazine is the natural substrate for Renilla luciferase. However, over a dozen of coelenterazine analogs have been synthesized, now commercially available from Interchim. These coelenterazine analogs all function as substrates for Renilla luciferase with different properties in terms of emission wavelength, cell membrane permeability and quantum efficiency.



Coelenterazine also emits light from enzyme-independent oxidation (autoluminescence), enhanced by superoxide anion and peroxynitrite in cells and tissues. Through the use of a specially designed coelenterazine derivative and buffer formulation, the Renilla Luciferase Assay Kit yields reliable, linear results with minimal autoluminescence background and superior sensitivity.

Description	P/N :	Qty
Renilla Luciferase Assay Kit	<b>FP-BE7930</b>	150 tests
	<b>FP-BE7931</b>	1000 tests
Kit contents : Coelenterazine, Renilla Luciferase Lysis Buffer, Renilla Luciferase Assay Buffer, Renilla Luciferase Assay Enhancer		

### Related substrates :

See "Coelenterazines", as stand alone products

## ■ Renilla Mullerei Luciferase, recombinant

Description	P/N :	Qty
Renilla Mullerei Luciferase, 95% purity (more than $8 \times 10^{14}$ Qps/mg)	<b>FP-BX6710</b>	1 mg

## ■ Renilla Mullerei Luciferase, pUC19 plasmid (pRLuc)

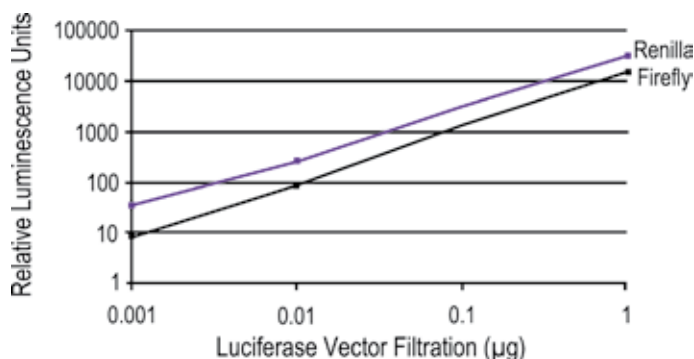
These protein uses coelenterazine and coelenterazine derivatives as substrate.  
The Renilla protein expresses well in bacteria.

Description	P/N :	Qty
pUC19 pRLuc	<b>FP-BS8180</b>	25 µg

## ■ Firefly and Renilla Luciferases Assay Kit

- **Sensitivity and Linearity** : Linear correlation between reporter gene expression and light output for transfection using 1 ng to 1 µg DNA of either Firefly or Renilla luciferase reporter construct.
- **Low Autoluminescence & High Sensitivity** : Reduced autoluminescence background for Renilla luciferase assay and consequently increased sensitivity.
- **Convenient** : One kit for both luciferase assays.

Firefly and Renilla luciferases are widely used as reporter genes for studying gene regulation and function, and for pharmaceutical screening. Renilla Luciferase is often used in conjunction with Firefly Luciferase as a normalizing transfection control or for multiplex transcriptional reporter assays. As with many enzymes, Firefly luciferase and Renilla luciferase follow Michaelis-Menten kinetics and thus maximum light output is not achieved until substrates (above the  $K_m$ ) and co-factor are present in large excess. When assayed under these conditions, light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules. Our Firefly & Renilla luciferase assay kit is designed for detection and quantification of Firefly and Renilla luciferase reporter enzymes from cultured cells in a simple, efficient and linear fashion.



Description	P/N :	Qty
Firefly and Renilla Luciferases Assay Kit	FP-BE7810	10 ml
	FP-BE7811	100 ml

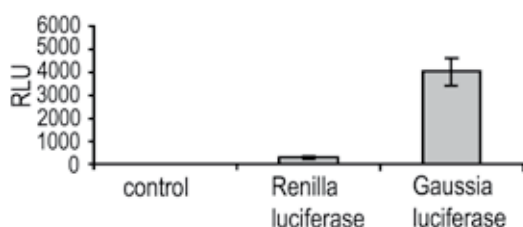
Kit contents per 10 ml : 2 x 1 mg D-Luciferin, 100 µL 100X Coelenterazine, 10 mL 5X Passive Lysis Buffer, 10 mL Firefly Luciferase Assay Buffer, 10 mL Renilla Luciferase Assay Buffer, 10 mL Renilla Luciferase Assay Enhancer.



## ■ Humanized Gaussia Luciferase

*Gaussia* luciferase, a novel reporter for gene expression, is the smallest and brightest known luciferase. Recommended for studying weak promoters, hard-to-transfect cells and HTS applications.

- ▶ **Greater brightness** : *Gaussia* luciferase expressed in mammalian cells is as much as 750-fold brighter than native *Renilla reniformis* luciferase
- ▶ **Avoid cell lysis** : *Gaussia* luciferase with secretion signal is secreted into the media. It is therefore necessary to only assay supernatants for enzyme activity without the need for lysing the cells. Considerable time is saved since time course experiments can be performed sampling the same group of transfected cells without lysing the cell
- ▶ **Extremely stable** to elevated temperature : up to 60°C and approx. 20% recovery following a 15 minute incubation at 99°C
- ▶ pH resistance : surviving a **pH range of 3-11**
- ▶ Resistance to detergents : 1-5% non-ionic detergents (NP-40, Triton X-100, Triton X-114, CHAPSO), cholate, deoxycholate etc
- ▶ Ability to recover activity after treatment with 7M guanidine chloride or 8M urea + NP-40



*Gaussia* luciferase uses coelenterazine and its derivatives to catalyse the oxidative decarboxylation of coelenterazine to produce coelenteramide and light. It has an emission spectral peak at 480 nm.

The specific activity of this luciferase in the presence of high concentrations of coelenterazine (10 µM) is extremely high :  $1.24 \times 10^{16}$  Qps/mg (Quanta per second per milligram)

Description	P/N :	Qty
pGluc-basic-1 promoter-less with secretion signal A promoterless vector with a MCS site upstream of the humanized <i>Gaussia</i> luciferase coding sequence (with secretion signal). This vector is designed for promoter analysis and will express secreted <i>Gaussia</i> luciferase. The transfected cells can be reused for multiple sampling.	<b>BU2550</b>	25 µg
pCMV-Gluc-1 positive control with secretion signal This positive control vector is very useful in evaluating the efficiency of transgene expression using <i>Gaussia</i> luciferase as a reporter. This vector has both Ampicillin resistance and Neomycin resistance. Therefore it can be easily propagated in <i>E. coli</i> and can be used to establish stable cell lines expressing <i>Gaussia</i> luciferase.	<b>BS8160</b>	25 µg
pCMV-KDEL-Basic-1 for intracellular expression	<b>BU2570</b>	25 µg
pCMV-KDEL-Gluc-1 for intracellular expression	<b>BU2560</b>	25 µg
<b>Related products :</b> Anti- <i>Gaussia</i> Luciferase, rabbit titer >1:10000 UptiFectin-On transfection reagent	<b>CJ3430</b> <b>CK5060</b>	250 µl 0.5 ml

## ■ Gaussia Luciferase Assay kit

The *Gaussia* luciferase assay kit **stabilizes the flash signal** emitted by the *Gaussia* luciferase thus making it possible to use it as a reporter gene for high throughput applications

The humanized *Gaussia* luciferase is secreted into the culture media and only the media needs to be assayed by the addition of native coelenterazine.

The *Gaussia* Assay Reagent (GAR) is prepared freshly by diluting the coelenterazine stock with the assay buffer. The assay is performed as following :

- Add 50µl of GAR to 20 µl *Gaussia* luciferase sample from microtiter or culture well samples
- Mix well and read in luminometer

Description	P/N :	Qty
Gaussia Luciferase Assay kit	<b>FP-BY7160</b> <b>FP-BY7161</b>	5 ml (100 tests) 50 ml (1000 tests)
Kit contains : pre-dissolved coelenterazine (100X concentration) and an assay buffer with stabilizers (increase emission up to 45 minutes).		



Coelenterazine – coelenterate luciferin – is the substrate for a number of marine bioluminescent enzymes, including those from marine organisms *Renilla*, *Gaussia*, *Pleuromamma* (**luciferases**) *Aequorea* (**aequorin**) and *Obelia* (**obelin**). In some of these reactions it is utilized as a simple substrate being catalytically turned over in the bioluminescent reaction catalyzed by luciferases, while in others, such as aequorin or obelin, it is incorporated as part of the photoprotein.

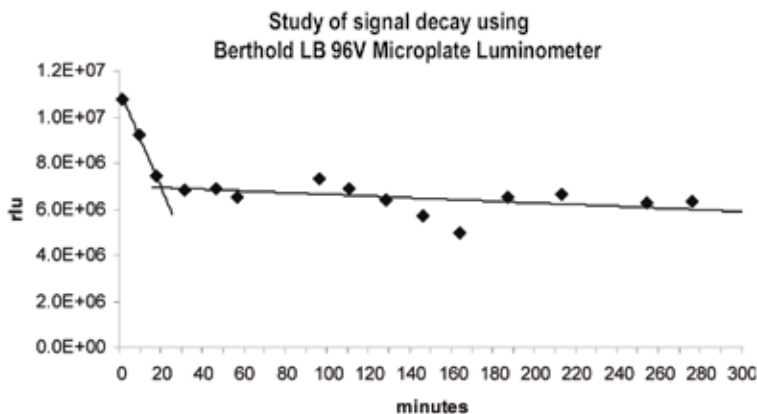
## ■ Coelenterazine, native

The native coelenterazine, the luminophore of the native aequorin complex, is the standard substrate used in many applications using luciferase reporter assays. Bioluminescent detection of calcium concentration is highly sensitive in a broad concentration range (0.1  $\mu$ M to >100  $\mu$ M)<sup>1-4</sup>. Monitoring of reporter genes (phot gene and luc gene) using coelenterazine is also a major application. Other uses of coelenterazine include bioluminescence resonance energy transfert (BRET)<sup>5,10</sup> and chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues<sup>6-9</sup>.

Coelenterazine native is recommended when a fast regeneration is important.

### References

- 1) Meth. Cell Biol. 40, 305(1994);
- 2) Meth. Enzymol. 172, 164, (1989);
- 3) J. BioChem. 105, 473(1989);
- 4) J. Chem. Soc. Chem. Commun. 21, 1566(1986)
- 5) Meth. Enzymol. 57, 271(1978);
- 6) Tetrahedron Lett. 31, 2963(1973);
- 7) Nature 256, 236(1975);
- 8) Anal. BioChem. 219, 169(1994);
- 9) proc. Natl. Acad. Sci. U SA 96, 151(1999);
- 10) Molecular Pharmacology, 70:1802-1811 (2006)



This result prompts to prepare FRESH Coelenterazine working solution and then to let it sit for 15-20 minutes at room temperature before use in order to achieve best accurate and high sensitivity.

Description	P/N :	Qty
Coelenterazine, native	UP972331	50 $\mu$ g
Highest purity	FP-97233B	250 $\mu$ g
	UP972333	1 mg
	UP972334	10 mg

Please contact us for bulk quote at [interbiotech@interchim.com](mailto:interbiotech@interchim.com)

Also available : Coelenterazine native for in vivo applications #FP-BV0730.

## ■ Coelenterazine 400a

Protein interactions study in BRET with GFP acceptor

Coelenterazine 400a, also known as DeepBlue™ C, is a coelenterazine derivative that serves as a substrate for *Renilla luciferase* (Rluc) and generates an emission peak centered around 400 nm. It is the preferred Rluc substrate for BRET studies because it has minimal interference with the emission of the GFP acceptor (GFP vectors are presented in the Bioscience Innovation catalog).

Description	P/N :	Qty
Coelenterazine 400a	UPBB8391	50 $\mu$ g
	FP-BB839B	250 $\mu$ g
	UPBB8392	1 mg

See other coelenterazines in the Bioscience Innovation catalog.



## ■ Coelenterazine H (Benzyl-Coelenterazine)

For calcium assay in vitro or protein interactions study in BRET with YFP acceptor

Coelenterazine H works better with Calcium activated photoproteins (Aequorin, obelin) compared to native Coelenterazine; however this is true only *in vitro*. This cell membrane-permeable, very sensitive, specific, intracellular luminophore is useful for measuring changes in  $Ca^{2+}$  i.e. in cells that have been transfected with apoaquorin cDNA. In this system, coelenterazine is required for the regeneration of aequorin, a protein that emits light in the presence of calcium, from apoaquorin produced in cells. The luminescence intensity appears to be directly proportional to the  $Ca^{2+}$  concentration. Coelenterazine-H exhibits an approximate 16-fold greater luminescence intensity (emission max. : ~ 466 nm ; half-time total of 0.6 - 1.2 sec.) as compared to the native Coelenterazine. Has been used to measure intracellular  $Ca^{2+}$  signals in *Dictyostelium discoideum* chemotaxis and in plant wound healing.

The bioluminescence resonance energy transfer (BRET) method, between Renilla luciferase and a variant of GFP, the yellow fluorescent protein (YFP) allows real-time detection of protein-protein interactions in vivo.

Description	P/N :	Qty
Coelenterazine H	<b>UPR30782</b>	50 µg
	<b>FP-R3078B</b>	250 µg
	<b>UPR30783</b>	1 mg
	<b>UPR30784</b>	10 mg

### Related product :

BAPTA, AM	<b>FP-486103</b>	25 mg
	<b>FP-486104</b>	20 x 1 mg

This  $Ca^{2+}$  chelator that delays the peak and increases the duration of light emission from aequorin

Also available : Coelenterazine H for in vivo #FP-BV0680

## ■ Other coelenterazines available

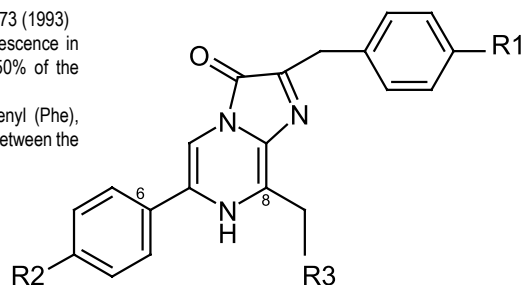
Table of Luminescent Properties of Coelenterazine Products with Apoaquorin\*  
See complete descriptions in the Bioscience Innovation catalog.

Coelenterazine	P/N :	R1 <sup>(2)</sup> #	R2 <sup>(6)</sup> #	R3 <sup>(8)</sup> #	λ max. Emission (nm)	Relative Luminescence capacité §	Relative Intensity §	Half-rise Time(s) §
Coelenterazine Native	<b>FP-97233A</b>	OH	OH	Phe	465	1.00	1.00	0.4-0.8
Coelenterazine cp	<b>FP-R3079A</b>	OH	OH	CP	442	0.95	20	0.15-0.3
Coelenterazine f	<b>FP-43876A</b>	F	OH	Phe	473	0.80	18	0.4-0.8
Coelenterazine fcp	<b>FP-R4711A</b>	F	OH	CP	452	0.57	135	0.4-0.8
Coelenterazine h	<b>FP-R3078A</b>	H	OH	Phe	464	0.82	10	0.4-0.8
Coelenterazine hcp	<b>FP-08353A</b>	H	OH	CP	444	0.67	190	0.15-0.3
Coelenterazine i	<b>FP-R3080A</b>	I	OH	Phe	476	0.70	0.03	8
Coelenterazine ip	<b>FP-R4712A</b>	I	OH	2P	441	0.54	47	1
Coelenterazine n	<b>FP-39819A</b>	Naph	OH	Phe	467	0.26	0.01	5

\* All datas from BioChem. J. 261, 913(1989) (other data can be found in O.Shimomura Cell Calcium 14, 373 (1993)

§ Luminescence capacity is the total light emission of aequorin in saturating  $Ca^{2+}$ . Intensity of luminescence in saturating  $Ca^{2+}$  measured at max emission wavelength. Half-rise time is the delay elapsed to get 50% of the maximum emission.

# substituant groups R1, R2 and R3, in positions 2, 6 and 8, are hydrogen (H), hydroxyl (OH), Phenyl (Phe), CycloPentyl (CP), 2-propionyl (2P), Naphthyl (Naph), methyl (Met). Coelenterazine e has a -CH<sub>2</sub>CH bridge between the 6-phenyl-OH and position 2 of the imidazopyrazinone core.



The popular reporter gene, luc gene, encodes the Firefly luciferase. The ATP-dependent oxidation of the substrate D-luciferin by oxygen produces an emission centered around 562 nm. The light output is proportional to luciferase concentration when both D-luciferin and ATP exist in large excess.

Interchim supplies D-luciferin in various forms : free acid, potassium salt and sodium salt and derivatives with acetoxymethyl (AM), methyl ether and DMNPE. The potassium and sodium salt forms are the most popular because they are readily water-soluble. The potassium salt is also the form used in live animal assay. Interchim's D-Luciferins are strictly controlled via several chemical analyses and also via a final enzyme assay to ensure consistency.

Description	P/N :	Qty
D-Luciferin, free acid, >99,0%	<b>FP-27060A</b>	25 mg
	<b>FP-27060B</b>	100 mg
	<b>FP-27060C</b>	250 mg
	<b>FP-27060D</b>	1 g
D-Luciferin, K <sup>+</sup> salt, >99,0%	<b>FP-M1224A</b>	25 mg
Potassium salt is the recommend salt form for in vivo uses.	<b>FP-M1224B</b>	50 mg
	<b>FP-M1224C</b>	500 mg
	<b>FP-M1224D</b>	1 g
D-Luciferin, Na <sup>2+</sup> salt, >99,0%	<b>FP-726045</b>	10 mg
	<b>FP-72604A</b>	25 mg
	<b>FP-72604B</b>	50 mg
	<b>FP-72604C</b>	1 g
D-Luciferin AM, cell permeant	<b>FP-M1909A</b>	5 mg
The cell-permeant D-luciferin AM ester enters easily into live cells, and is well retained once it is cleaved by intracellular esterases to D-luciferin.		
D-Luciferin ethyl ether	<b>FP-CF4421</b>	10 mg
Cell permeant analog with 30% higher signal intensity		
DMNPE-caged D-Luciferin	<b>FP-21639A</b>	5 mg
DMNPE-caged D-luciferin is a D-luciferin ester derivative which can cross cell membranes efficiently. Once inside the cells, the ester is continuously hydrolyzed to a supply of D-luciferin. Alternatively a burst of D-luciferin is generated by UV photolysis.		
References : Luque-Ortega J.R. et al. - In Vivo Monitoring of Intracellular ATP Levels in Leishmania donovani Promastigotes as a Rapid Method To Screen Drugs Targeting Bioenergetic Metabolism, Antimicrobial Agents and Chemotherapy, p. 1121-1125, Vol. 45, No. 4 (2001)		
D-Luciferin 6-methyl ether, Na salt	<b>FP-M1418A</b>	10 mg
D-Luciferin methyl ether has been proposed to be a substrate for microsomal dealkylase/cytochrome P450. Demethylation of the substrate generates D-luciferin, and thus can be detected via bioluminescence with extremely high sensitivity.		
Reference : Denburg J. et al. Substrate-binding properties of firefly luciferase I. Luciferin-binding site. Archs Biochem. Biophys. 134, 381-394 (1969).		
D-Luciferin-6-0-β-D-galactopyranoside	<b>FP-CQ6410</b>	5 mg
β-Galactosidase substrate		
Reference : Yang, Y. et al., Homogeneous enzyme immunoassay modified for application to luminescence-based biosensors, Anal. Biochem 33: 102-107 (2005)		

# Substrates for glucosidases reporter systems (β-galactosidase, β-glucuronidase, β-glucosidase)



Int

## ■ β-Galactosidase fluorescent substrates sampler Kit

This kit consists of samples of several of our most popular galactosidase substrates and their reference fluorophores allowing multiplexed analysis of lacZ β-Galactosidase activity at a variety of wavelengths. This kit is perfect for those occasions where the preferred wavelength of detection is under development.

Description	P/N :	Qty
β-Galactosidase substrates sampler Kit	<b>FP-BM8400</b>	1 kit
Contains :		
Subst. FDG	#52476A, 10 mg	
Fluo.Std. Fluorescein	#193659, 10 mg	
Subst. Res-Gal	#524739, 10 mg	
Fluo.Std. Resorufin	#95432A, 10 mg	
Subst. TFMU-Gal	#M11419, 10 mg	
Fluo.Std. TFMU	#434769, 10 mg	

Each substrate is available separately, and also many other derivatives (inhibitors, activators,...). Please inquire.

## ■ Other colorimetric substrates for glycosidases

Description	P/N :	Qty
o-NPG (o-Nitrophenyl-β-D-galactopyranoside, MW : 301.3 ; $\lambda_{abs}$ (cleaved) : 420 nm)	<b>UP556683</b>	5 g
X-GLU (5-Bromo-4-Chloro-3-Indolyl-β-D-Glucopyranoside, MW : 408.6)	<b>UP193325</b>	100 mg
X-GAL (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside, MW : 408.6)	<b>UP40534M</b>	1 g