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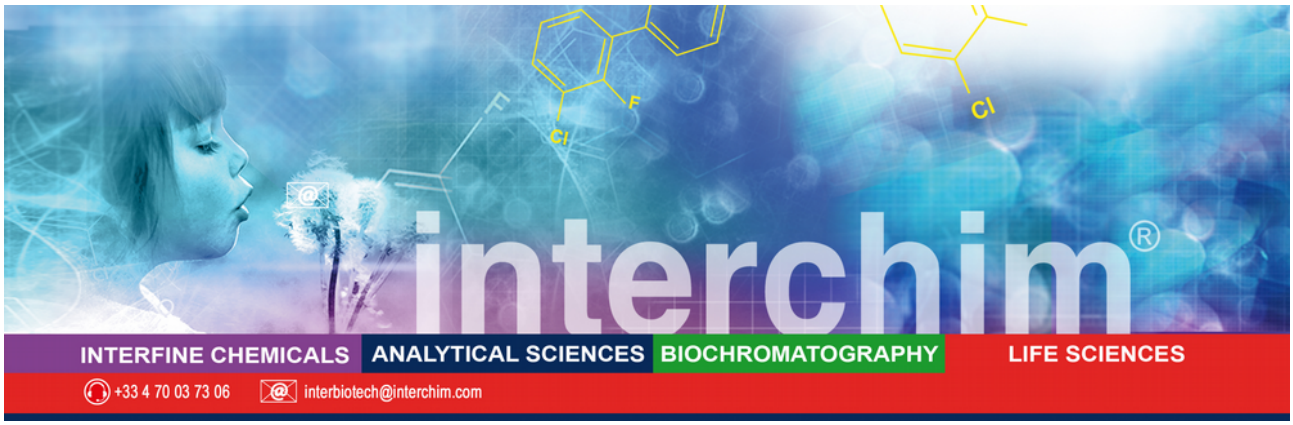

Coelenterazines

Bioluminescent substrates for bioassays (Ca^{2+} measurements, gene reporter assays, ROS assays)

Product Information

Product cat.number *	R1 £	R2 £	R3 £	λ max. Emission (nm)	Relative Luminescence capacity §	Relative Intensity § Half-rise Time (s) § *(ms) §§*	
Coelenterazine native FP-97233B, 250µg *	OH	OH	Phe	465	1.00	1.00	0.4-0.8 *6-30ms*
Coelenterazine cp FP-R3079B, 250µg *	OH	OH	CP	442	0.95 *0.63*	20 28	0.15-0.3 *5-5ms*
Coelenterazine e FP-T8677B, 250µg *	OH	OH#	Phe	405 and 465	0.5	4	0.15-0.3
Coelenterazine f FP-43876B, 250µg *	F	OH	Phe	473	0.80 *0.80*	18 20	0.4-0.8 *6-30ms*
Coelenterazine fcp FP-R4711B, 250µg *	F	OH	CP	452	0.57	135	0.4-0.8
Coelenterazine h FP-R3078B, 250µg * Ultra Pure FP-RK5440, 1mg	H	OH	Phe	464	0.82 0.75	10 *16*	0.4-0.8 *6-30ms*
Coelenterazine hcp FP-08353B, 250µg *	H	OH	CP	444	0.67 *0.65*	190 *500*	0.15-0.3 *2-5ms*
Coelenterazine i FP-R3080B, 250µg *	I	OH	Phe	476	0.70	0.03	8
Coelenterazine ip FP-R4712B, 250µg *	I	OH	2P	441	0.54	47	1
Coelenterazine n FP-39819B, 250µg *	Naph	OH	Phe	467	0.26 *0.25*	0.01 *0.15*	5 *6-30ms*
Coelenterazine 2-methyl FP-T8889B, 1 mg				N/A	N/A	N/A	N/A
Coelenterazine 400a FP-BB839B, 250µg *				~400	N/A	N/A	N/A
Sample kit FP-42176C, 9 x 25 µg							

*:other quantities on inquire (50 µg , 1mg, bulk)

£: Substituents groups R1, R2 and R3, in positions 2, 6 and 8, are hydrogen (H), hydroxyl (OH), Phenyl (Phe), CycloPentyl (CP), 2-propionyl (2P), Napthyl (Naph), methyl (Met)

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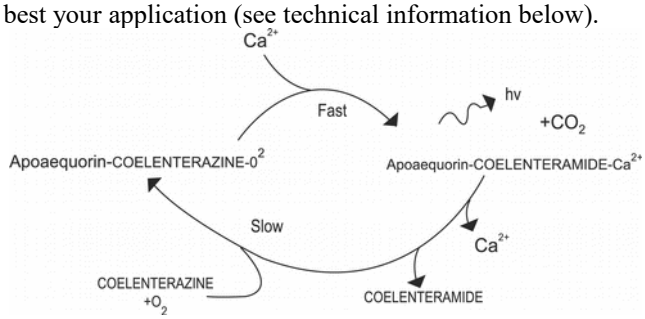
[#] Coelenterazine e has a -CH2CH- bridge between the 6-phenyl-OH and position 2 of the imidazopyrazinone core.

§ Data from BioChem. J. 261, 913(1989) [normal characters]
§§ Data from O.Shimoraura in Cell Calcium 14, 373 (1993) for calcium measurements [*smaller size and italic characters*]

Introduction to Bioluminescence / Coelenterazines

Bioluminescence is generation of light by a biochemical reaction involving oxidation of a substrate via an enzyme. This phenomenon has been used extensively in different formats for life science research and drug discovery owing to its extremely high sensitivity, replacing advantagely hazardous methods as radioelement.

Coelenterazine (native form) is a bioluminescent substrate for enzymes apoaequorin and Renilla luciferase. It's structure has been investigated [ref]. FluoProbes® offer include a large selection of coelenterazine analogs, with each of them giving unique luminescent properties (table below). Careful selection of a coelenterazine derivative may be necessary to suit at best your application (see technical information below).



The involved enzymatic complex (~22Kda) contains apoaequorin protein (a protein from *Aequorea victoria* jelly fish and other marine organisms), molecular oxygen, and the luminogen coelenterazine. It releases carbon dioxide and blue light (465 nm) upon oxidation in presence of Ca^{2+}.

The **apoaequorin / Coelenterazine** system has shown privileged advantages for bioassays, notably for measurements of Ca^{2+} and of Reactive Oxygen Species (ROS).
Examples of bioluminescence applications include:
- Calcium detection in live cells or tissues ([1-7]) +
- Superoxide and peroxynitrite anion detection (ROS) ([10-14])
- Reporter assays ([8]): aequorin has been largely used to tag (by plasmid or other engineering methods) recombinent proteins, and then monitor their expression (localization, regulation...). +
- ELISA, bioluminescence resonance energy transfer (BRET) for protein interaction studies ([9])
- Drug high throughput screening.

Directions for use

Handling and Storage

All coelenterazines are soluble in MeOH or EtOH; DO NOT DISSOLVE IN DMSO. Solution may be stored frozen, protected from light, preferably under dry nitrogen.
Solubility in water in rather low. To avoid adverse effects from methanol solutions, aqueous solutions >1mM can be prepared pH 7 buffer containing 50 mM 2-hydroxypropyl-cyclodextrin.(see also ADVASEP and FluoCD™ technology).

Guidelines for use – Ca measurements

Using coelenterazine / aequorin complex for calcium measurement

Coelenterazine analogs provide various affinities of Ca^{2+} complexation, and different spectral properties. Compared with fluorescent calcium indicators, aequaporin complex/Coelenterazine system has several advantages in detecting calcium :

- The Ca2+/aequorin complex can detect a broad range of calcium concentrations, from ~0.1 µM to >100 µM,
- Background is lower than with fluorescence, and no autofluorescence of sample occurs,
- Although signal is lower, higher signal/noise ratios can be obtained with imaging equipments,
- The aequorin complex is not exported from cells, allowing to follow calcium concentration changes in cells for hours to days.

Coelenterazine cp, f, h, hcp and n have been used for these applications. Especially, Coelenterazine hcp shows a 500 fold enhancement of light intensity (and thus improved Ca^{2+} sensitivity) over the native Coelenterazine. Coelenterazine *e* has two emission peaks at 405 and 465 nm, respectively, making it possible to measure calcium concentration via the ratio of emission intensities [17].

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Using coelenterazine / aequorin complex for injection

Sterile Coelenterazine and Benzyl Coelenterazine Luciferin have been processed by chromatography and ultra-filtration and are supplied prepackaged in pyrogen free injection vials under sterile conditions.

The supplied vial of diluents contains one milliliter of a 50/50 mixture of USP grade Ethanol and USP grade Propylene Glycol. Using your hands to warm the Propylene Glycol will decrease the time it takes to dissolve the Luciferin but do not be alarmed if this longer than expected.

In our shelf life studies 100% Propylene Glycol(PG) is an excellent solvent by itself, even if it takes a considerable amount of time to dissolve the Luciferin. PG retards the auto-oxidation of the Luciferin which occurs quite rapidly once the Luciferin is dissolved in Methanol or Ethanol. This 50/50 diluent was designed to solvate the Luciferin rapidly, prolong the storage life, and is far less inflammatory to small vessels so that repeated venous access may occur.

Using coelenterazine / aequorin complex reporter assays

The development of aequoporin vectors prompted many gene reporter assays, as well aequaporin tagged recombinant protein reporter assays. Recombinant proteins can even be targeted in definite cell compartments, for fine measurements.

FluoProbes® offer the largest choice of synthetic coelenterazine derivatives that allow users to choose the ones that suit at best their specific applications.
All these coelenterazine analogs are highly purified (purity : >98%) to ensure optimal results in bioassays.

Guidelines for use – BRET

For BRET[2] application with Coelenterazine 400a (FP-BB839B), that is hardly soluble in water, stock solution can be made in ethanol at 1mM (or ~0.4mg/ml) concentration and stored at –20°C in the dark. Avoid using DMSO to dissolve the material as it may cause oxidation.
Prepare a 20-fold (50µM) dilution of the stock solution with the recommended buffer [3,.9] we recommend Dulbecco's phosphate buffered saline containing $CaCl_2$ (0.1g/l), $MgCl_2.6H_2O$ (0.1g/l) and D-glucose(1g/l) supplemented with aprotinin (2µg/ml). The working solution is susceptible to oxidation by air, thus should not be stored.
See reference (9)

For BRET [1] application with Coelenterazine H (FP-R3078B), please send reference (24).

Guidelines for use – ROS

See references (11, 13)
See related products: chromogenic probes (NBT, MTT), fluorescent and luminescent probes (MCLA, Coelenterazine,... cf FT-38544).

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Technical information

Coelenterazine (native) is the luminophore of the native aequorin complex and also the substrate for Renilla luciferase. Bioluminescent detection of calcium concentration is highly sensitive in a broad concentration range (0.1µM to >100µM) [1-4]. 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)[5] and chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues[6-9].

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Coelenterazine Native FP-97233B, 250 µg * $C_{26}H_{21}N_3O_3$; MW: 423.5; (M)	The standard substrate widely used in many applications. Coelenterazine native is recommended when a fast regeneration is important.
Coelenterazine cp FP-R3079B 250 µg * $C_{28}H_{23}N_3O_3$; MW: 415.5; (M)	Coelenterazine *cp* aequorin complex generates luminescence intensity 15 times higher and has a faster response time to calcium than native coelenterazine does (20.21.22).
Coelenterazine e FP-T8677B, 250 µg * $C_{25}H_{25}N_3O_3$; MW: 449.5; (M)	Coelenterazine *e* has the highest rate in the *in vitro* regeneration of aequorin, with two emission peaks at 405 and 465 nm, respectively. This make it possible to measure calcium concentration via the ratio of emission intensities in the range of pCa 5-7 [17], improving measurement accuracy (correction for variation of coelenterazine concentration). However it do not suit intracellular applications because of poor permeability[19]. Is was also found less stable in solution[19]. Structure data
Coelenterazine f FP-43876B, 250 µg * $C_{26}H_{20}N_3O_2F$; MW:457.5; (M)	Coelenterazine *f* with aequorin complex gives an almost 20 times higher luminescence intensity than that of native coelenterazine while its emission maximum is about 8 nm longer. Coelenterazine *f* has been found the most cell permeant (19). It is recommended when a high Ca^{2+} sensitivity of regenerated aequorin is needed (20.21.22).
Coelenterazine fcp FP-R4711B, 250 µg * $C_{25}H_{24}FN_3O_2$; MW:417.5; (M)	Coelenterazine *fcp* is a synthetic derivative of coelenterazine. Its luminescence intensity is 135 times higher than that of native coelenterazine (20.21.22).
Coelenterazine h FP-R3078B, 250 µg * $C_{26}H_{21}N_3O_2$; MW: 407.5; (M)	Its luminescence intensity is more than 10 times higher than that of aequorin complex formed from native coelenterazine. Coelenterazine *h* is also more sensitive to Ca^{2+}. It is also used for reporter assays (Blood, 94.6, 1999, 1899-1905, Blanpain C Article)
Coelenterazine h, sterile FP-BV0680, 250 µg FP-BV0681, 500 µg FP-BV0682, 1 mg $C_{26}H_{21}N_3O_2$; MW: 407.5; **(M)**	Benzyl-Coelenterazine (Coelenterazine H) in sterile injection vials for animal *in vivo* imaging only.
Coelenterazine hcp FP-08353B, 250 µg * $C_{25}H_{25}N_3O_2$; MW: 399.5; (M)	It's luminescence intensity is the highest (190 times than that of aequorin complex formed from native coelenterazine) with a fast response time to calcium (20.21.22).
Coelenterazine i FP-R3080B, 250 µg * $C_{26}H_{20}IN_3O_2$; MW:533.4; (M)	Its luminescence intensity is almost 50 times higher than that of native coelenterazine while its response time to calcium is much slower than the latter (20.21.22).
Coelenterazine ip FP-R4712B, 250 µg * $C_{23}H_{23}N_3O_3$; MW: 389.5; (M))	Its luminescence intensity is more than 10 times higher than that of aequorin complex formed from native coelenterazine.
Coelenterazine n FP-39819B, 1 mg * $C_{30}H_{23}N_3O_3$; MW: 457.5; (M)	Its luminescence intensity is the weakest of all coelenterazine analogs and its response time to calcium is also much slower than that of native Coelenterazine. Coelenterazine *n* is reported to be a very useful low-sensitivity coelenterazine.
2-methyl Coelenterazin FP-T8889B, 1 mg * Coelenterazine 2-methyl analog $C_{20}H_{17}N_3O_2$; MW: 331.4; (M)	Methyl coelenterazine has been reported to be a superior antioxidant for cells against reactive oxygen species (ROS) such as singlet oxygen and superoxide anion [14]. It is membrane-permeant, nontoxic and highly reactive toward ROS (potent antioxidant). As oxidative stress is believed to be a mediator o apoptosis, methyl coelenterazine should be important tool for apoptosis study. Biochem. Pharmacol. 60, 471 (2000) ;Immunol. Today 15, 7 (1994) ;Anal. Biochem. 206, 273 (1992) ; Circ. Res. 84, 1203 (1999) Soluble in MeOH or EtOH
Coelenterazine 400a FP-BB839B, 250 µg * $C_{26}H_{21}N_3O$; MW: 391.5; (M)	Coelenterazine 400a, also called DeepBlueC is a coelenterazine derivative that serves as a substrate for a Renilla luciferase (Rluc) and generates an emission peak centered around 400nm. It is the best Rluc substrate for BRET studies because it has minimal interference with the emission of GFP acceptor. See guidelines for BRET application +.
Coelenterazine Sampler Kit FP-42176C, 1 kit (9x25µg) ; (M)	contains 25µg each of nine coelenterazine analogs: native coelenterazine, coelenterazine *cp*, coelenterazine *f*, coelenterazine *fcp*, coelenterazine *h*, coelenterazine *hcp*, coelenterazine *i*, coelenterazine *ip*, and coelenterazine *n*.

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*:other quantities on inquire (50 µg , 1mg, bulk)

Related products

- UptiFectin-On Transfection Reagent, CK5060
- D-Luciferin (also a luminescent substrate, used for gene reporter assays with Firefly luciferase) FP-M1224A
- Luciferase assay kits with luminescent substrate in solution, FP-BX0320
- MCLA, ROS probes, FP-38544A

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Catalog size quantities and prices may be found at http://www.fluoprobes.com
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