

Coelenterazines

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

Product Information

Product name cat.number	MW	R1	R2	R3	λ max. Emission (nm)	Relative Luminescence capacity §	Relative Intensity §	Half-rise Time (s) § (ms) §§
Coelenterazine Native UP972333, 1 mg	423.50	OH	OH	Phe	465	1.00	1.00	0.4-0.8 6-30ms
Coelenterazine 2-methyl UPT88891, 1 mg	331.37				N/A	N/A	N/A	N/A
Coelenterazine 400a UPBB8392, 1 mg	391.46				~400	N/A	N/A	N/A
Coelenterazine cp UPR30791, 50 μg	415.48	OH	OH	CP	442	0.95 0.63	20 28	0.15-0.3 5-5ms
Coelenterazine e UPT86770, 50 μg	449.50	OH	OH [#]	Phe	405 and 465	0.5	4	0.15-0.3
Coelenterazine f UP438761, 50 μg	425.45	F	OH	Phe	473	0.80 0.80	18 20	0.4-0.8 6-30ms
Coelenterazine fcp UPR47110, 50 μg	417.48	F	OH	CP	452	0.57	135	0.4-0.8
Coelenterazine h UPR30783, 1 mg	407.50	H	OH	Phe	464	0.82 0.75	10 16	0.4-0.8 6-30ms
Coelenterazine hcp UP083532, 50 μg	399.49	H	OH	CP	444	0.67 0.65	190 500	0.15-0.3 2-5ms
Coelenterazine i UPR30801, 50 μg	533.36	I	OH	Phe	476	0.70	0.03	8
Coelenterazine ip UPR47120, 50 μg	389.45	I	OH	2P	441	0.54	47	1
Coelenterazine n UP398192, 50 μg	457.52	Naph	OH	Phe	467	0.26 0.25	0.01 0.15	5 6-30ms
Sample kit UP42176C, 9x25 μg								

Substituent 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 -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.Shimomura 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 advantageously hazardous methods as radioelement.

Coelenterazine (native form) is a bioluminescent substrate for enzymes apoaquorin and *Renilla* luciferase. Its structure has been investigated^{ref}. Uptima® offer include a large selection of coelenterazine analogs, with each of them giving unique luminescent properties (table above). Careful selection of a coelenterazine derivative may be necessary to suit at best your application (see technical information below).

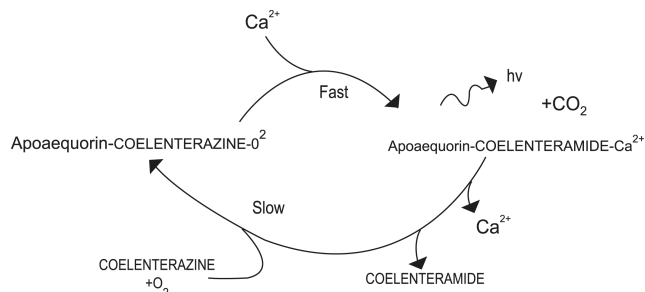
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The involved enzymatic complex (~22Kda) contains apoaequorin protein (a protein from *Aequorea victoria* jelly fish and other marine organisms), molecular oxygen, and the luminogen coelenterazine. It releases carbon dioxide and blue light (465 nm)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



The **apoaquorin / 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) recombinant proteins, and then monitor their expression (localization, regulation...). [+](#)
- ELISA, bioluminescence resonance energy transfer (BRET) for protein interaction studies ^(9, 23)
- 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 is 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).

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, aequorin complex/Coelenterazine system has several advantages in detecting calcium :

- The Ca^{2+} /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.

Using coelenterazine / aequorin complex reporter assays

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

Uptima® 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.

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Guidelines for use – BRET

Standard BRET¹ uses coelenterazine H ([UPR30783](#)), that allows transfer of energy to YFP acceptor ([25](#)).

For BRET² application with Coelenterazine 400a ([UPBB8392](#)), the substrate for *Renilla luciferase* generates an emission peak centered around 400 nm. It is the preferred Rluc substrate for BRET² studies with the GFP acceptor.²³ Coelenterazine 400a 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 CaCl₂ (0.1g/l), MgCl₂.6H₂O (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](#))

Guidelines for use – ROS

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 The coelenterazine derivative is membrane-permeant, nontoxic and highly reactive toward ROS. As oxidative stress is believed to be a mediator of apoptosis,¹² methyl coelenterazine should be another important tool for apoptosis study.

See references ([11](#), [13](#))

See related products: chromogenic probes (NBT, MTT), fluorescent and luminescent probes (MCLA, Coelenterazine,... cf FT-[38544](#)).

References

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Technical information

Coelenterazine Native $C_{26}H_{21}N_3O_3$; MW: 423.5; (M)	UP972331, 50 µg UP972333, 1 mg	The standard substrate widely used in many applications. Coelenterazine 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) ^{5,23} and chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues ⁶⁻⁹ . Coelenterazine native is recommended when a fast regeneration is important.
Coelenterazine cp $C_{28}H_{23}N_3O_3$; MW: 415.48; (M)	UPR30791, 50 µg UPR30793, 1 mg	Coelenterazine <i>cp</i> 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 $C_{25}H_{25}N_3O_3$; MW: 449.5; (M)	UPT86770, 50 µg FP-T8677B, 250 µg	Coelenterazine <i>e</i> has the highest rate in the <i>in vitro</i> 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

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Coelenterazine f C ₂₆ H ₂₀ N ₃ O ₂ F; MW:457.45 (M)	UP438761, 50 µg UP438763, 1 mg	Coelenterazine <i>f</i> 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 <i>f</i> 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 C ₂₅ H ₂₄ N ₃ O ₂ ; MW:417.5 (M)	UPR47110, 50 µg UPR47111, 1 mg	Coelenterazine <i>fcp</i> is a synthetic derivative of coelenterazine. Its luminescence intensity is 135 times higher than that of native coelenterazine ^(20,21,22) .
Coelenterazine h C ₂₆ H ₂₁ N ₃ O ₂ ; MW: 407.5 (M)	UPR30782, 50 µg UPR30783, 1 mg	Its luminescence intensity is more than 10 times higher than that of aequorin complex formed from native coelenterazine. Coelenterazine <i>h</i> is also more sensitive to Ca ²⁺ . It is also used for reporter assays (Blood, 94.6, 1999, 1899-1905, Blanpain C Article)
Coelenterazine hcp C ₂₅ H ₂₅ N ₃ O ₂ ; MW:: 399.5 (M)	UP083532, 50 µg UP083534, 250 µg	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 C ₂₆ H ₂₀ IN ₃ O ₂ ; MW:533.4 (M)	UPR30801, 50 µg UPR30803, 1 mg	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 C ₂₃ H ₂₃ N ₃ O ₃ ; MW: 389.5 (M)	UPR47120, 50 µg UPR47122, 1 mg	Its luminescence intensity is more than 10 times higher than that of aequorin complex formed from native coelenterazine.
Coelenterazine n C ₃₀ H ₂₃ N ₃ O ₃ ; MW: 457.5 (M)	UP398192, 50 µg UP398193, 1 mg	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 <i>n</i> is reported to be a very useful low-sensitivity coelenterazine.
2-methyl Coelenterazin Coelenterazine 2-methyl analog C ₂₀ H ₁₇ N ₃ O ₂ ; MW: 331.4 (M)	UPT88890, 50 µg UPT88891, 1 mg	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. Soluble in MeOH or EtOH Biochem. Pharmacol. 60, 471 (2000) ;Immunol. Today 15, 7 (1994) ;Anal. Biochem. 206, 273 (1992) ; Circ. Res. 84, 1203 (1999)
Coelenterazine 400a C ₂₆ H ₂₁ N ₃ O; MW: 391.5 (M)	UPBB8391, 50 µg UPBB8392, 1 mg	Coelenterazine 400a, also called DeepBlue C 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	UP 42176C, 1 kit (9x25µg)	contains 25µg each of nine coelenterazine analogs: native coelenterazine, coelenterazine <i>cp</i> , coelenterazine <i>f</i> , coelenterazine <i>fcp</i> , coelenterazine <i>h</i> , coelenterazine <i>hcp</i> , coelenterazine <i>i</i> , coelenterazine <i>ip</i> , and coelenterazine <i>n</i> .

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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