

## Coelenterazines

### Product Information

Coelenterazines are **bioluminescent substrates for bioassays** (Ca<sup>2+</sup> measurements, gene reporter assays, ROS assays)

Coelenterazine (native form) is a bioluminescent substrate for enzymes apoaequorin and Renilla luciferase (a 22kD complex, purified from jellyfish and other marine organisms). FluoProbes offer includes 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 best suit your application (see also technical information below).

All these coelenterazine analogs are highly purified (purity : >98%) to ensure optimal results in bioassays.

Coelenterazine	Item. #	MW	$\lambda$ max. Emission (nm)	Relative Luminescence capacity §	Relative Intensity §	Half-rise Time (s) § (ms) §§
Coelenterazine Native	97233	423.50	465	1.00	1.00	0.4-0.8 6-30ms
Coelenterazine cp	R3079	415.48	442	0.95 <i>0.63</i>	20 28	0.15-0.3 5-5ms
Coelenterazine e	T8677	449.50	405 and 465	0.5	4	0.15-0.3
Coelenterazine f	43876	425.45	473	0.80 <i>0.80</i>	18 20	0.4-0.8 6-30ms
Coelenterazine fcp	R4711	417.48	452	0.57	135	0.4-0.8
Coelenterazine h	R3078	407.50	464	0.82 <i>0.75</i>	10 16	0.4-0.8 6-30ms
Coelenterazine hcp	08353	399.49	444	0.67 <i>0.65</i>	190 500	0.15-0.3 2-5ms
Coelenterazine i	R3080	533.36	476	0.70	0.03	8
Coelenterazine ip	R4712	389.45	441	0.54	47	1
Coelenterazine n	39819	457.52	467	0.26 <i>0.25</i>	0.01 <i>0.15</i>	5 6-30ms
Coelenterazine 2-methyl	T8889	331.37	N/A	N/A	N/A	N/A
Coelenterazine 400a	BB839	391.46	N/A	N/A	N/A	N/A
Sampler Kit	42176					

§ All data from BioChem. J. 261, 913(1989) [normal characters]

§§ All datas from *O.Shimomura in Cell Calcium 14, 373 (1993 for calcium measurements [in characters in smaller size and italic]*

### Introduction to Bioluminescence / Coelenterazines

**Bioluminescence** is the light produced in a biochemical reaction involving the 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 and non-hazardous nature. Examples of bioluminescence applications include: -calcium detection in live cells or tissues (1-7) -reporter gene assays (8) -ELISA, bioluminescence resonance energy transfer (BRET) for protein interaction studies (9) -superoxide anion detection (10-14) -high throughput drug screening.

The **aequaporin / coelenterazin** system has shown privileged advantages for bioassays, notably Ca<sup>2+</sup> measurement, and ROS measurements. The involved complex, ca 22Kda, contains apoaequorin protein (a protein from *Aequorea victoria* jelly fish), molecular oxygen, and the luminogen coelenterazine. It releases carbon dioxide and blue light (465nm) upon oxidation in presence of Ca<sub>2+</sub>.

The development of aequaporin vectors prompted many gene reporter assays, as well aequaporin tagged recombinant protein reporter assays. Major applications are the investigation of Ca<sup>2+</sup> and reactive Oxygen species (ROS). Recombinant proteins can even be targeted in definite cell compartments, for fine measurements.

FluoProbes the largest choice synthetic coelenterazine derivatives that allow users to choose the ones that best suit the specific applications.

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## Applications and Directions for use

### General guidelines

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: 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 different affinity to Ca<sup>2+</sup> affinities to the complex, and different spectral properties. Compared with fluorescent calcium indicators, it has several advantages in detecting calcium:

- The Ca<sup>2+</sup>/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, making it possible 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<sup>2+</sup> 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.<sup>17</sup>

### Using coelenterazine / aequorin complex for ROS species

Coelenterazine products are widely used to detect superoxide and peroxynitrite via chemiluminescence<sup>(13,11)</sup>.

### Using coelenterazine / reporter assays

The development of aequaporin 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.

## References

- 1) Meth. Cell Biol. 40, 305(1994);
- 2) Meth. Enzymol. 172, 164, (1989);
- 3) J. BioChem. 105, 473(1989), [abstract](#);
- 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), [article](#);
- 10) Free Radic. Biol. Med. 28, 1232(2000);
- 11) Circ. Res. 84, 1203(1999), [article](#);
- 12) Immunol. Today 15, 7(1994);
- 13) Anal. BioChem. 206, 273(1992), [abstract](#);
- 14) BioChem. Pharmacol. 60, 471(2000);
- 15) J. Clin. Chem. Clin. BioChem. 25, 23(1987);
- 16) Bioluminescence and Chemiluminescence: Current Status (Stanley, P.E. and Kricka, L.J., Eds), pp.511-514, Wiley, Chichester;
- 17) BioChem. J. 251, 405(1988);
- 18) BioChem. Biophys. Res. Commun. 233, 349(1997).
- 19) Biochem. J. 326, 297±298 (1997), [Article](#)
- 20) Biochem. J. 261, 913(1989), [abstract](#)
- 21) Cell Calcium 12, 635(1991)
- 22) Cell Calcium, 14, 373 (1993)
- 23) Molecular Pharmacology, 70:1802-1811 (2006)
- 24) Agulhon C. *et al.*, J Physiology (2007), [abstract](#)
- 25) Hamdan F. *et al.*, J. Biol. Chem., in press (2007), [article](#)
- 26) Anal Biochem. 282(1):158-61 (2000) [Abstract](#) (Dual Luciferase Assay)
- 27) Anal Biochem. 356:94-99 (2006) [Abstract](#) (Dual Luciferase assay)

## Related products

- BTA-2 (Dual luciferase assay), [FP-QU6360](#)
- Coenzyme A, [627374](#)
- 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](#)
- Coelenterazines *in vivo* grade (for animal *in-vivo* imaging) [FP-BV073A](#)

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## Technical information

<b>Coelenterazine Native</b> FP-97233B, 250µg* $C_{26}H_{21}N_3O_3$ ; MW: 423.5; (M)	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) <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 transfer(BRET) <sup>5</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.
<b>Coelenterazine cp</b> FP-R3079B, 250µg* $C_{28}H_{23}N_3O_3$ ; 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 <sup>(20,21,22)</sup> .
<b>Coelenterazine e</b> FP-T8677B, 250µg* $C_{25}H_{25}N_3O_3$ ; MW: 449.5; (M)	Coelenterazine e has the highest rate in the generation in vitro of aequorin, with two emission peaks at 405 and 465 nm, respectively, making it possible to measure calcium concentration via the ratio of emission intensities in the range of pCa 5-7 <sup>17</sup> (improves accuracy because it is independent on coelenterazine concentration), however it do not suit intracellular applications because of poor permeability <sup>19</sup> . It was also found less stable in solution <sup>19</sup> . The ethylene bridge between C5 and the ortho position of the phenyl ring at C6, constrains the phenyl ring to be coplanar with the imidazopyrazinone ring.
<b>Coelenterazine f</b> FP-43876B, 250µg* $C_{26}H_{20}N_3O_2F$ ; MW:457.5; (M)	The luminescence intensity of this aequorin complex is almost 20 times higher than that of native coelenterazine while its emission maximum is about 8 nm longer than that of the latter. Coelenterazine f has been found the most cell permeant <sup>(19)</sup> ; it is recommended when a high Ca <sup>2+</sup> sensitivity of regenerated aequorin is needed <sup>(20,21,22)</sup> .
<b>Coelenterazine fcp</b> FP-R4711B, 250µg* $C_{25}H_{24}FN_3O_2$ ; MW:417.5; (M)	Coelenterazine fcp is a synthetic derivative of coelenterazine. Its luminescence intensity is 135 times higher than that of native coelenterazine <sup>(20,21,22)</sup> .
<b>Coelenterazine h</b> FP-R3078B, 250µg* $C_{26}H_{21}N_3O_2$ ; MW: 407.5; (M)	Luminescence intensity is more than 10 times higher than that of the latter. Used for reporter assay (Blood, 94.6, 1999, 1899-1905, Blanpain C <a href="#">Article</a> )
<b>Coelenterazine hcp</b> FP-08353B, 250µg* $C_{25}H_{25}N_3O_2$ ; MW:: 399.5; (M)	Luminescence intensity of its aequorin complex is 190 times higher than that of aequorin complex formed from native coelenterazine while the response time to calcium is faster <sup>(20,21,22)</sup> .
<b>Coelenterazine i</b> FP-R3080B, 250µg* $C_{26}H_{20}IN_3O_2$ ; MW:533.4; (M)	Luminescence intensity is almost 50 times higher than that of native coelenterazine while its response time to calcium is much slower than the latter <sup>(20,21,22)</sup> .
<b>Coelenterazine ip</b> FP-R4712B, 250µg* $C_{23}H_{23}N_3O_3$ ; MW: 389.5; (M)	Luminescence intensity is more than 10 times higher than that of the latter.
<b>Coelenterazine n</b> FP-39819B, 250µg* $C_{30}H_{23}N_3O_3$ ; MW: 457.5; (M)	Luminescence intensity is the weakest of all coelenterazine analogs and its response time to calcium is also much slower than that of native. Coelenterazine n is reported to be a very useful low-sensitivity coelenterazine.
<b>2-methyl Coelenterazin</b> FP-T8889B, 1mg* $C_{20}H_{17}N_3O_2$ ; 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. The coelenterazine derivative is membrane-permeant, nontoxic and highly reactive toward ROS. As oxidative stress is believed to be a mediator o apoptosis, methyl coelenterazine should be another important tool for apoptosis study. It is also a potent antioxidant D-Luciferin free acid. Biochem. Pharmacol. 60, 471 (2000)      Immunol. Today 15, 7 (1994) Anal. Biochem. 206, 273 (1992)      Circ. Res. 84, 1203 (1999)
<b>Coelenterazine 400a</b> FP-BB839B, 250g* $C_{26}H_{21}N_3O$ ; MW: 391.5; (M)	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 ± λ <sub>abs</sub> .424nm: 3.9
<b>Coelenterazine Sampler Kit</b> FP-42176C, 1 kit (9x25µg)	contains 25µg each of nine coelenterazine analogs: coelenterazine, coelenterazine cp, coelenterazine f, coelenterazine fcp, coelenterazine h, coelenterazine hcp, coelenterazine i, coelenterazine ip, and coelenterazine n.

For any question please contact FluoProbes – Interchim\_

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