

# Product Information

## Coelenterazine Analogs

Product	MW	Catalog No.	Size
Coelenterazine Sampler Kit ( <i>native</i> , <i>cp</i> , <i>f</i> , <i>hcp</i> , <i>h</i> , <i>hcp</i> , <i>i</i> , <i>ip</i> and <i>n</i> analogs)	---	10123	25 ug each
Aquaphile™ Coelenterazine ( <i>native</i> ), lyophilized solid	423.5	10126-50ug	50 ug
		10126	5 x 100 ug
Aquaphile™ Coelenterazine <i>h</i> , lyophilized solid	407.5	10127-50ug	50 ug
		10127	5 x 100 ug
Coelenterazine ( <i>native</i> )	423.5	10110	50 ug
		10110-2	250 ug
		10110-1	1 mg
Coelenterazine <i>h</i>	407.5	10111	50 ug
		10111-2	250 ug
		10111-1	1 mg
Coelenterazine <i>cp</i>	415.5	10112	50 ug
		10112-2	250 ug
		10112-1	1 mg
Coelenterazine <i>hcp</i>	399.5	10113	50 ug
		10113-2	250 ug
		10113-1	1 mg
Coelenterazine <i>f</i>	425.5	10114	50 ug
		10114-2	250 ug
		10114-1	1 mg
Coelenterazine <i>n</i>	457.5	10115	50 ug
		10115-2	250 ug
		10115-1	1 mg
Coelenterazine <i>ip</i>	389.5	10116	50 ug
		10116-2	250 ug
		10116-1	1 mg
Coelenterazine <i>hcp</i>	417.5	10117	50 ug
		10117-2	250 ug
		10117-1	1 mg
Coelenterazine <i>i</i>	533.4	10121	50 ug
		10121-2	250 ug
		10121-1	1 mg
Coelenterazine <i>e</i>	449.5	10124	50 ug
		10124-2	250 ug
Coelenterazine 400a (DeepBlueC™)	391.5	10125	50 ug
		10125-2	250 ug
		10125-1	1 mg
Methyl Coelenterazine	331.4	10122	50 ug
		10122-1	1 mg

## Storage and Handling

Store solids at -20°C or -70°C, protected from light, under nitrogen or argon for long-term storage. Product is stable for at least 3 years from date of receipt when stored as recommended.

For non-Aquaphile™ coelenterazines use methanol or ethanol according to the table below to prepare stock solutions (> 1 mg/mL). For Aquaphile™ coelenterazines use water or PBS. Do not use DMSO (dimethylsulfoxide), as coelenterazine analogs may be unstable in this solvent. Keep calcium-free when stored in solution and avoid using glass containers. Solutions of coelenterazine analogs are susceptible to oxidation by air. For best results, protect stock solutions from light and store at <-70°C under nitrogen or argon. Stock solutions can be further diluted with buffer to the appropriate working concentration. We recommend preparing fresh working solutions just before use to avoid precipitation and decomposition of coelenterazine analogs.

## Molecular Information

Analog	Abs/Em <sup>[a]</sup> (nm)	Color and Form	Solubility
Aquaphile™ ( <i>native</i> )	429/466	Yellow solid	water or PBS buffer
Aquaphile™ <i>h</i>	437/466	Yellow solid	water or PBS buffer
<i>native</i>	429/466	Yellow solid	MeOH or EtOH
<i>h</i>	437/466	Yellow solid	MeOH or EtOH
<i>cp</i>	430/442	Yellow solid	MeOH or EtOH
<i>hcp</i>	430/444	Yellow solid	MeOH or EtOH
<i>f</i>	437/473	Yellow solid	MeOH
<i>n</i>	431/467	Yellow solid	MeOH
<i>ip</i>	430/441	Yellow solid	MeOH
<i>hcp</i>	430/452	Yellow solid	MeOH
<i>i</i>	440/476	Yellow solid	MeOH or EtOH
<i>e</i>	---/405, 465	Brown solid	MeOH or EtOH
400a (DeepBlueC™)	---/400 <sup>[b]</sup>	Pink solid	1 mM (~0.4 mg/ml) in EtOH
<i>methyl</i>	---/---	Yellow solid	MeOH or EtOH

<sup>[a]</sup> Emission with apoaequorin; see Table 2 for luminescent properties with *Renilla* luciferase.

<sup>[b]</sup> Emission with *Renilla* luciferase for Coelenterazine 400a (DeepBlueC™).

## Product Description

Coelenterazine and its analogs are luminescent enzyme substrates for apoaequorin and *Renilla* luciferase. Apoaequorin is used as a calcium indicator whereas *Renilla* luciferase is commonly used as a reporter of transcription regulation. Coelenterazine binds to apoaequorin to form aequorin which emits light upon binding to calcium. Aequorin can be used to measure a broad concentration range of calcium from ~0.1 uM to >100 uM. *Renilla* luciferase has been widely used as a reporter protein and as a bioluminescence donor in bioluminescence resonance energy transfer (BRET) to study protein-protein interactions. Other uses of coelenterazine include chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues.

Biotium offers high purity native coelenterazine and a number of coelenterazine analogs with different properties in terms of emission wavelength, cell membrane permeability and quantum efficiency. Table 1 and Table 2 on page 2 summarize the luminescent properties of coelenterazine derivatives with apoaequorin and *Renilla* luciferase, respectively.

Aquaphile™ coelenterazines are water soluble formulations that can be readily dissolved in water or buffer for *in vivo* use. Aquaphile™ formulations are available for coelenterazine *native* (catalog no. 10126) and coelenterazine *h* (catalog no. 10127).

Coelenterazine 400a (DeepBlueC™) is a *Renilla* luciferase substrate with an emission peak centered at 400 nm. This substrate is most ideal for BRET studies because it has minimal interference with the emission of the GFP acceptor. BRET studies are typically performed in Dulbecco's Phosphate Buffered Saline (DPBS) with calcium and magnesium supplemented with Aprotinin (2 ug/mL), using a final concentration of 50 uM substrate.

Methyl coelenterazine (2-methyl analog) has been reported to be a potent antioxidant 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 of apoptosis, 2-methyl coelenterazine may be a useful tool for apoptosis studies.

The Coelenterazine Sampler Kit (catalog no. 10123) contains 25 ug each of nine coelenterazine analogs (*native*, *cp*, *f*, *hcp*, *h*, *hcp*, *i*, *ip* and *n*).

## References

- 1) *Meth. Cell Biol.* 40, 305(1994).
- 2) *Meth. Enzymol.* 172, 164(1989).
- 3) *J. Cell Biol.* 115, 1259(1991).
- 4) *Cell Calcium*, 14, 373(1993).
- 5) *Proc. Natl. Acad. Sci. USA* 96, 151(1999).
- 6) *Proc. Natl. Acad. Sci. USA* 97, (7), 3684 (2000).
- 7) *Free Radic. Biol. Med.* 28, 1232(2000).
- 8) *Circ. Res.* 84, 1203(1999).
- 9) *Immunol. Today* 15, 7(1994).
- 10) *Anal. Biochem.* 206, 273(1992).
- 11) *Biochem. Biophys. Res. Commun.* 233, 349(1997).
- 12) *Mol Imaging*, 3 (1), 43(2004).
- 13) *Biochem. Pharmacol.* 60, 471(2000).
- 14) *Biochem. J.* 261, 913(1989).
- 15) *Cell Calcium*, 12, 635(1991).

**Table 1. Luminescent Properties of Coelenterazine Analogs with Apoaequorin\***

Coelenterazine analog	Emission maximum (nm)	Relative Luminescence capacity	Relative intensity	Half-rise time (s)
native	466	1.00	1.00	0.4-0.8
<i>cp</i>	442	0.95	15	0.15-0.3
<i>e</i>	405, 465	0.5	4	0.15-0.3
<i>f</i>	473	0.8	18	0.4-0.8
<i>hcp</i>	452	0.57	135	0.4-0.8
<i>h</i>	466	0.82	10	0.4-0.8
<i>hcp</i>	444	0.67	190	0.15-0.3
<i>i</i>	476	0.70	0.03	8
<i>ip</i>	441	0.54	47	1
<i>n</i>	467	0.26	0.01	5

\*All data from *Biochem. J.* 261, 913(1989).

**Table 2. Luminescent Properties of Coelenterazine Analogs with *Renilla* Luciferase\*\***

Coelenterazine analog	Emission maximum (nm)	Total light (%)	Initial intensity (%)
native	475	100	100
<i>cp</i>	470	23	16
<i>e</i>	418, 475	137	750
<i>f</i>	473	28	58
<i>h</i>	475	41	57
<i>n</i>	475	47	68

\*\* All data from *Biochem. Biophys. Res. Commun.* 233, 349(1997).

## Related Products

Catalog number	Product
41024-4L	Water, Ultrapure Molecular Biology Grade
22020	10X Phosphate-Buffered Saline (PBS)
30028-L	Steady-Luc™ Firefly HTS Assay Kit (Lyophilized)
30075	Firefly Luciferase Assay Kit (Lyophilized)
30085	Firefly Luciferase Assay Kit 2.0
30082	<i>Renilla</i> Luciferase Assay Kit 2.0
30081	Firefly & <i>Renilla</i> Luciferase Single Tube Assay Kit
10100	D-Luciferin, Free Acid
10101	D-Luciferin, Potassium Salt
10102	D-Luciferin, Sodium Salt

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including our flash-type Firefly and *Renilla* luciferase kits, D-luciferin formulations, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

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