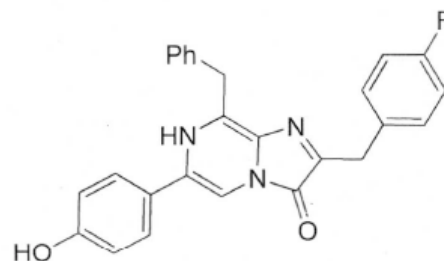


How To Use:

Cat# 345 NF-CTZ-F- FB Coelenterazine-F CAS# 123437-16-1 MW = 425.5

General Notes and Properties:

Coelenterazine-F (CTZ-F) differs only from natural Coelenterazine by replacement of the hydroxyl group on the R-1 phenol group by fluorine. This semisynthetic coelenterazine was first synthesized by Dr Osamu Shimomura in his studies stemming from the discovery of Aequorin calcium activated photoprotein from *Aequoria victoria* at Friday Harbor Marine Station in the late 1960s.



Solubility:

CTZ-F is not soluble in water and is not stable in contact with air, water, or any oxidizing agents. Avoid dissolving in DMSO because unknown and unstudied reactions occur, leading to rapid degradation and unstudied degradation products.

CTZ-F is soluble in alcohols, methanol and least toxic solvent is 100% Ethanol and 0.1-1 mg/ml solutions can be made then these are added to your buffer of choice, and advise keeping the buffers as below pH 7.0 as possible because alkaline conditions will rapidly degrade and sometimes immediately precipitate most Coelenterazine analogs.

Storage:

Best mix the product from the dry solid state with ethanol prior to use, and then mix with your buffer. Please allow it to stabilize (auto-oxidize partially) in the aqueous buffer for 20-30 minutes before in vitro assays. This mixture should provide several hours of working time with slightly diminishing light emission over time (3-4 hours at room temperature). Many people “get away” with mixing CTZ-F and storing it in Ethanol at -20C to -70C however this and any Coelenterazine analog are high energy dioxetanone ring structures that will spontaneously decompose even at low temperatures.

Special Properties of CTZ-F vs. Natural Coelenterazine:

When CTZ-F is used with apo-aequorin to make the activated partially oxidized Coelenterazine metastable complex, the resultant complex only produces 80% of the total number of photons in comparison to natural Coelenterazine. The advantages of CTZ-F: namely requiring much less time to generate the Aequorin complex, which is generally a very slow step, (40% complete in 60 minutes with native Coelenterazine). However due to the large rapid emission of light upon contact with Calcium ions, the complex produces nearly 20 times more signal with the same rise time and more less identical emission spectra as natural Coelenterazine.

Coelenterazine analog differences:

Calcium activated apo-proteins require oxygen and reducing environments and time to make the slow forming complex of apo-aequorin or apo-obelin and a meta-stable semi-oxidized Coelenterazine which will emit light rapidly and spontaneously when three free calcium ions attach to the EF hand domains of the complex. The complex must be formed in the absolute absence of Calcium ions or these proteins act as slow luciferases and emit light continuously.

Coelenterazine analogs as RENILLA LUCIFERASE substrates:

Cat.	Coelenterazine Derivate	Emission Maximum (nm)	Total Light (%)	Initial Intensity (%)
#303	native	475	100	100
#335	e-CTZ	418 and 475	137	750
#345	f-CTZ	473	28	58
#301	h-CTZ	475	41	57
#340	400a (DeepBlueC™)	400	3	n.a.

*data based on *Biochem. Biophys. Res. Commun.* **233**, 349(1997)

Coelenterazine analogs as AEQUORIN substrates:

Cat.	Coelenterazine Derivate	Emission Maximum (nm)	Relative Luminescence capacity	Relative Intensity
#303	native	466	1.00	1.00
#335	e-CTZ	405/465	0.5	4
#345	f-CTZ	473	0.80	18
#301	h-CTZ	466	0.82	10
#340	400a (DeepBlueC™)			

*data based on *Biochem. J.* **261**, 913(1989)

References:

Cause of spectral variation in the luminescence of semisynthetic aequorins
By Osamu Shimomura
Link: <http://www.ncbi.nlm.nih.gov/pubmed/7887908?dopt=Abstract>

Structure–function studies on the active site of the coelenterazine-dependent luciferase from *Renilla* by Jongchan Woo, Matthew H. Howell, and Albrecht G. von Arnim
Link: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2271170/>

Demonstration of Improvements to the Bioluminescence Resonance Energy Transfer (BRET) Technology for the Monitoring of G Protein–Coupled Receptors in Live Cells
Link: <http://jbx.sagepub.com/content/13/9/888>