

# Product Data Sheet

## Renilla mullerei Luciferase

Catalog Number: 0309

### Product Description

*Renilla mullerei* is a species of soft coral that is characterized by a green fluorescence. The fluorescence is the result of a three protein energy transfer system that generates light via the oxidation of coelenterazine. The luciferase enzyme is a Ca-independent monooxygenase, EC 1.13.12.5, that emits blue light ( $\lambda_{max}$  485 nm) in the presence of reduced coelenterazine and oxygen to yield coelenteramide and CO<sub>2</sub>. Rm-Luc can accept most coelenterazine analogs as substrates.

### Product Specifications

Protein	1mg (0309-1), 300µg (0309-2)
Long-term Storage	2 years at -80°C Aliquot to avoid repeated freezing and thawing
Short-term Storage	1 month at 4°C
Formulation	In: 10 mM Na <sub>2</sub> HPO <sub>4</sub> , 140 mM NaCl, 2 mM KH <sub>2</sub> PO <sub>4</sub> , 3 mM KCl, 20% glycerol, pH 7.6
Molecular Weight	36 kDa by SDS-PAGE 36 kDa

### Technical Information

Length	331 aa
Molecular Weight	36,110
Molar Extinction Coefficient	62640
Isoelectric Point	5.98
Excitation	NA
Emission	485 nm

### Instructions for Use

- The amount of luciferase used for a given assay should be empirically determined by titrating the enzyme. The amount of luciferase that gives the highest signal: noise ratio should be selected.
- Prepare a coelenterazine stock by dissolving the powder in 1mL of acidified methanol (50 µL concentrated HCl to 10 mL of anhydrous methanol) to give a 200 µM solution. Aliquot and store at -80°C for up to 4 weeks. A working solution of coelenterazine is prepared by diluting the stock solution to 10 µM in 18 Mohm water.

**Important Notice:** Coelenterazine is unstable in aqueous solutions. It is recommended that working solutions are prepared daily. Activity results will vary depending on concentration of Coelenterazine used.

- For performing luciferase assays, dilute the enzyme in 50 mM Tris-Cl, 0.1 M NaCl, 10 mM MgSO<sub>4</sub>, 0.01% gelatin pH 7.5 to the desired RFU per assay. Add coelenterazine to 90 nM and immediately measure the light production at 485 nm with a 5-10 s integration.

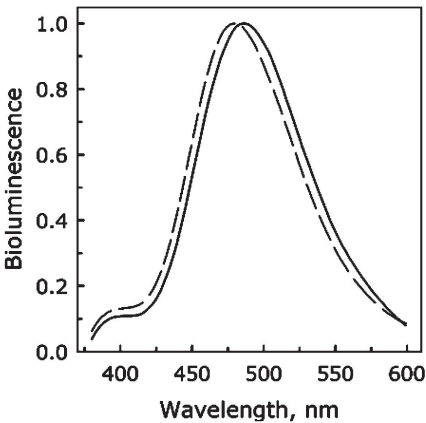


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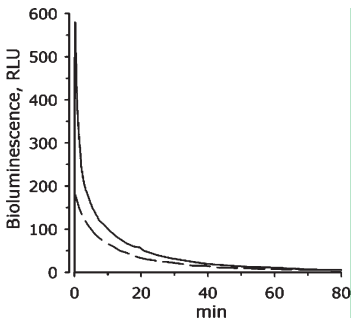
a division of Athena Environmental Sciences, Inc.

Athena Enzyme Systems™  
1450 South Rolling Road  
Baltimore, MD 21227  
USA

T (MD): 410-455-6319  
T (USA): 888-892-8408  
F: 410-455-1155  
aesinfo@athenaes.com



**Figure 1.** The emission spectrum for *Renilla mullerei* luciferase with free coelenterazine (solid line) and in the presence of the coelenterazine binding protein (dashed line). (From Titushin et al. 2008.)



**Figure 2.** The bioluminescent signal of *R. muelleri* luciferase in the presence of 91 nM coelenterazine (dashed line) and CBP (solid line). The luciferase concentration was 14 nM in 0.1M NaCl, 10mM MgSO<sub>4</sub>, 0.01% gelatin, 50 mM Tris-HCl pH 7.5, a condition which gives optimum bioluminescence activity of *R. muelleri* luciferase with coelenterazine. (From Titushin et al. 2008.)

### Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTIC OR VETERINARY USE. Handle using standard laboratory practices for personal protection. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information, see complete Material Safety Data Sheet.

### References

Titushin, M. S., Markova, S. V., Frank, L. A., Malikova, N. P., Stepanyuk, J. L and Vysotski, E. S. 2008. Coelenterazine-binding protein of *Renilla mulleri*: cDNA cloning, overexpression and characterization as a substrate of luciferase. Photochem. Photobiol. Sci. 7:189-196.

Bryan, B. J., Szent-Gyorgyi, C. 2001. Luciferases, fluorescent proteins, nucleic acids encoding the luciferases and fluorescent proteins and the use thereof in diagnostics, high throughput screening and novelty items. U.S. Patent No. 6,232,107.