Product Data Sheet

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Renilla mullerei Luciferase

Catalog Number: 0309

Product Description

Renilla mullerei is a species of soft coral that is characterized by a green fluorescence. The green fluorescent protein obtained from this organism exhibits nearly symmetrical excitation and emission peaks with a relatively small Stokes shift. (Labas et al., 2002) This is in contrast to the more commonly employed GFP from *Aequorea victoria* which has two wavelengths at which excitation occurs, and a broader emission spectra. The monomer extinction coefficient for Rr-GFP is about 5.5-fold higher than the *Aequorea* GFP which, in conjunction with a slightly higher quantum yield, produces a brighter fluorescence. Recombinant *Renilla reniformis* GFP has the same level of fluorescence as the native protein.(Bryan et al., 2001) 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 (λmax485 nm) in the presence of reduced coelenterazine and oxygen to yield coelenteramide and CO2. 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-tem Storage	1 month at 4°C
Formulation	In: 10mM Na_2HPO_4, 140 mM NaCl, 2 mM KH_2PO_4, 3mM KCl, 20% glycerol, pH 7.6
Molecular Weight	26kDa by SDS-PAGE 22kDa

Technical Information

Length	331 aa
Molecular Weight	36,110
Molar Extinction Coefficient	62640
Isoelectric Point	5.98
Excitation	NA
Emission	480 nm

Instructions for Use

1. The amount of luciferase used for a given assay should be empirically determined by titering the enzyme. The amount of luciferase that gives the highest signal: noise ratio should be selected.

2. Prepare a colenterazine 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.*

3. For performing luciferase assays, dilute the enzyme in 50 mM Tris-Cl, 0.1 M NaCl, 10 mM $MgSO_4$, 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.

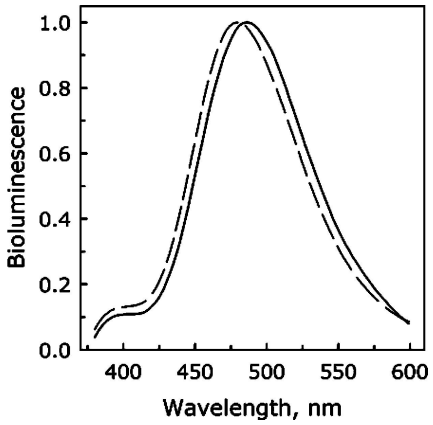

Figure 1. The emission spectrum for *Renilla mullerei* luciferase with free coelenterazine (solid line) and in the presence of the coelenterazine binding protein (dashed line). (From Titushin et al. 2008.)

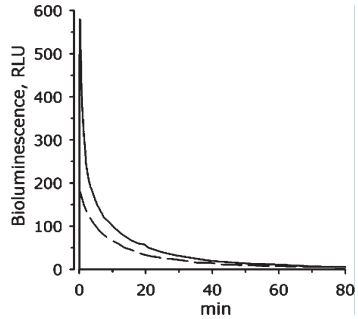

Figure 2. The bioluminescent signal of *R. muelleri* luciferase in the presence of 91 nM coelenterazine (dashed line) and CBP (solid line). The luciferase concentration was 14 nM in 0.1M NaCl, 10mM MgSO4, 0.01% gelatin, 50 mM Tris–HCl pH 7.5, a condition which gives optimum bioluminescence activity of *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 usingstandard laboratory practices for personal protection. Do not ingest, swallow or inhale. Do not get in eyes, on skin, ore on clothing. Wash thoroughly after handling. For complete safety information, see complete Material Safety Data Sheet.

References

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

Labas, Y. A., Gurskaya, N. G., Yanushevich, Y. G., Fradkov, A. F., Lukyanov, K. A., Lukyanov, S. A., and Matz, M. V. 2002. Diversity and evolution of the green fl uorescent protein family. Proc. Natl. Acad. Sci. USA. 99:4256-4261.

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.