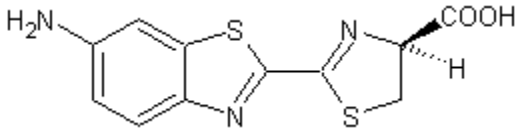
D-Aminoluciferin

Ordering Information:	*Storage Conditions:*
Product Number: 13415 (10 mg)	Keep at -20 °C and desiccated Expiration date is 12 months from the date of receipt

Chemical and Physical Properties



Molecular Weight: 279.34
Appearance: Light yellow powder
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Luminescence = 560 nm.

Biological Applications

Aminoluciferin reacts with luciferases to generate luminescence in the presence of ATP and $MgCl_2$. In the course of luciferase reactions photones are emitted, i.e., in the course of the reaction with the enzyme of the fire-fly Photinus pyralis at 605 nm and in the course of the reaction with the enzyme of the fire-fly Photinus plathiophthalamus at 549 or 570 nm, or wavelength corresponding to the used liciferin/luciferase system, respectively. The emission at 549 nm takes place if the enzyme originates from the dorsal organ of the fire-fly mentioned whereas the emission at 570 nm takes place if the enzyme originates from the ventral organ.

Aminoluciferin can be luminometrically detected even in the smallest concentrations since aminoluciferin is an excellent substrate for luciferase. In addition, any modification of the amino or carboxy group of aminoluciferin makes the resulted aminolucferin derivatives insensitive to luciferase reactions. These properties make the enzyme-cleavable group-modified aminolucierins excellent sensors for detecting enzyme activities by luminescence. Rapid and sensitive assays of proteolytic activity are important for general characterization of proteases and high-throughput screening for protease inhibitors. However, the inherent background of fluorescence, particularly in cell-based systems, can limit assay sensitivity. Moreover, to achieve maximum sensitivity, lengthy incubations are often required for accumulating the fluorescent assay product. Luminescent assays can often provide greater sensitivity in less time.

Aminoluciferin-based protease substrates provide an improved method for the sensitive monitoring of protease activity in purified preparations comprising the protease, in cell lysates or cells, either prokaryotic or eukaryotic cells. Preferred eukaryotic cells include mammalian cells, for example, human, feline, bovine, canine, caprine, ovine, swine, equine, non-human primate, e.g., simian, avian, plant or insect cells. The protease is detected using an amino-modified aminoluciferin or a carboxy-terminal protected derivative thereof, which modification comprises a substrate for the protease. The substrate, which comprises one or more amino acid residues which include the recognition site for the protease, is covalently linked to the amino group of aminoluciferin or the carboxy-terminal modified derivative via a peptide bond.

In the absence of the appropriate protease, a mixture comprising a substrate and luciferase will generate minimal light as minimal aminoluciferin is present (a small amount of light may be generated due to spontaneous hydrolysis of the peptide bond). In the presence of the appropriate enzyme, the peptide bond

linking the substrate and aminoluciferin (the bond immediately adjacent to the 6' position on the luciferin core molecule) can be cleaved by the protease to yield aminoluciferin, a substrate for luciferase. Thus, in the presence of luciferase, for instance, a native, a recombinant or a mutant luciferase, light is generated, which is proportional to the amount or activity of the protease. Any beetle luciferase, preferably a thermostable luciferase, may be employed in the method.

The aminoluciferin-based substrates are extremely sensitive substrates, only very small amounts of a biological sample (e.g., cells, and physiological fluids, blood, urine, etc., which comprise cells) are required to perform the assay. For example, using such an assay, the activity of caspase 3, caspase 7 and trypsin was found to be below the level of detection of a corresponding assay using a Rhodamine-110 caspase substrate although rhodamine-110 is considered to be one of the most sensitive indicators.

References

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