Luciferases are enzymes that catalyse light-emitting reactions in living organisms - bioluminescence. They occur in several species of firefly and in many species of bacterium. Firefly Luciferases are extracted by differential centrifugation and purified by gel filtration. Lyophilised luciferase with added stabilizer keeps for several months at -4°C.

Luciferins are substrates of luciferases. Firefly luciferin emits at 562 nm on reaction with oxygen, catalysed by luciferase in the presence of adenosine triphosphate (ATP) and magnesium ions, emission being directly proportional to luciferin concentration over the range 0.01-1000 nmol dm$^{-3}$. The ATP dependence of firefly luciferin bioluminescence is exploited in many ATP determinations and assays for the products of enzymatic reactions that utilize or produce ATP, e.g., kinases and substances involved in reactions catalysed by them.

The crustacean *Cypridina hilgendorfii* has a luciferin of very different chemical structure, but the mechanism of its bioluminescence is the same as that of the firefly except that no co-factor is required. Analogues of Cypridina luciferin have also been synthesised and used to detect superoxide of pathological origin. Scavengers of superoxide radicals, e.g., tea leaf catechins, quench Cypridina chemiluminescence, enabling their antioxidant activities to be conveniently measured.

![Figure B3.1](image.png)

**Figure B3.1**: Principle of bacterial bioluminescence, in which light is emitted by the oxidation of a long-chain fatty aldehyde by flavine mononucleotide, which is regenerated in a coupled reaction. NAD(P)H, nicotinamide adenine dinucleotide (phosphate; FMN, flavine mononucleotide.

Luminous bacteria are found widely in marine environments. Bacterial luciferase, which acts in accordance with the outline mechanism shown in Figure B3.1, does not have a luciferin substrate as such. Instead the light emission comes from a complex of luciferase, flavine mononucleotide and a long-chain fatty aldehyde$^{[1]}$. Thus bacterial bioluminescence is associated with a pyridine nucleotide rather than with the adenine nucleotide involved in firefly bioluminescence.