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Product Information

Firefly Lantern Extract

Catalog Numbers **FLE250 and F3641**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Heat-stable luciferin and the labile enzyme luciferase, responsible for light production by firefly tails, were isolated and purified in 1947.[1] It was also shown adenosine triphosphate (ATP) was required for the process. The overall reaction proceeds as follows:

$$\text{ATP + Luciferin + } O_2 \xrightarrow[\text{Magnesium Ions}]{\text{Firefly Luciferase}}$$

Oxyluciferin + Adenosine 5′-Monophosphate (AMP) + CO_2 + Pyrophosphate + Light

Highly sensitive photomultipliers permit the detection of extremely low concentrations of existing ATP or ATP as it forms in kinetic systems. The assay can be used to determine biomass because of an approximately equal distribution of ATP in all living matter. It is also used as an indicator for coupled reactions, which produce ATP as an end product.

Due to the unique sensitivity of the luciferase reaction, great care must be exercised to avoid microbial (biomass) or other trace ATP contamination. The rapid flare and decay of the light flash requires consistent timing of the light measurement to obtain dependable quantitation.

The intensity and duration of the light emitted are a function of reaction conditions, reagent purity, and other factors. Instruments ranging from a simple spectrophotometer to a bioluminometer may be employed for light measurement.

Table 1.
Firefly Lantern Extract Products

Catalog Number	Description
F3641	Firefly Lantern Extract in glycine buffer with added luciferin. Solutions are prepared by the addition of distilled water. When the 5 mg and 50 mg package sizes* are reconstituted with 5 ml and 50 ml, respectively, the following solution is obtained: 50 mM glycine, 1 mg/ml Firefly Lantern Extract, 0.15 mM luciferin, 0.1 mM Trizma® base, 10 mM magnesium sulfate, 0.5 mM EDTA, 1 mg/ml bovine serum albumin, and 0.1 mg/ml sodium azide. *Package sizes indicate the amount of dried firefly lanterns that have been extracted.
FLE250	Firefly Lantern Extract - A crude luciferin and luciferase. Each vial contains soluble extract from 250 mg of dried lanterns. After reconstituting with 25 ml of water, each vial will contain 0.06 M potassium phosphate and 0.02 M $MgSO_4$, pH 7.4

Precautions and Disclaimer

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Preparation of ATP Solutions

ATP Stock Solution - Sigma offers an ATP Standard, Catalog Number FLAAS, which contains 2 μmole of ATP. An ATP Stock Solution may be prepared by reconstituting one vial of the ATP Standard with 2.0 ml of distilled water, free of microbial contamination, to yield a 1 mM solution. Store the stock solution at –20 °C.

ATP Standard Solutions - Prepared by making serial dilutions of an aliquot of the ATP Stock Solution with distilled water. The extent of the dilutions depends upon the assay sensitivity desired. An ATP standard curve generated from 3 ATP Standard Solutions of different concentrations will generally suffice. The dilutions remain active up to 8 hours when stored on ice.

Sample Preparation

ATP can be extracted from most biological samples by suspending the sample in buffer (0.02 M glycine, 0.05 M Mg^{2+}, 0.004 M EDTA, pH 7.4) and heating for 45 seconds in a boiling water bath (100 °C). Alternatively ATP can be extracted by treating the sample with perchloric acid. The perchlorate supernatant must be neutralized prior to assay. ATP can also be extracted from mammalian cells using 0.05–0.1% TRITON® X-100.

Detergent extraction of ATP from bacterial cells requires a 0.02% quaternary ammonium salt such as benzalkonium chloride. The nonionic detergent, TRITON X-100, can lyse mammalian cells; however, it may not suitably lyse bacterial cell walls. Various agents, which have been reported to be useful for extraction of bacterial ATP, include Tris-EDTA, trichloroacetic acid, perchloric acid, ethanol, butanol, chloroform and DMSO.[2-5] Each carries advantages and disadvantages for the ATP extraction process and subsequent bioluminescent assay.

The choice of extraction technique is governed by the purpose of the study and influenced by practical considerations.[2] The investigator may find it beneficial to empirically determine the most appropriate extraction technique for the assay sensitivity desired.

Storage/Stability

Store products desiccated at –20 °C. Firefly Lantern Extract solutions remain active for ~2 weeks when stored refrigerated at 2–8 °C. Luciferase reagents should be protected from light. Vigorous agitation is to be avoided, as it may denature the luciferase enzyme.

Procedure

Bioluminescent Determination of ATP using Firefly Lantern Extract Solutions

The Firefly Lantern Extract contains voluminous amounts of luciferase, but less than optimal amounts of luciferin. Without fortification, the assay is capable of detecting 10^{-6} to 10^{-11} moles of ATP under the described assay conditions. The detection limit may be extended to 10^{-15} moles ATP with the addition of luciferin to a concentration of ~1 mM.

Notes: Arsenate ion is particularly inhibitory to the bioluminescent reaction. However, it is sometimes used to prolong the light emission period at the expense of sensitivity. A concentration of 0.02 M is suggested if arsenate is to be used for this purpose.

Optimal temperature for the luciferase-luciferin reaction is 28 °C. The reaction slows at higher temperatures.

Interferences - Contamination from any biosource (fingerprints, bacteria, etc.) must be avoided.

1. Prepare an appropriate Firefly Lantern Extract Solution in distilled water.
2. Zero the instrument employed.
   Note: For greater accuracy, convenience, and sensitivity, a bioluminometer is recommended. Instruments other than bioluminometers used for bioluminescence measurement include scintillation counters, fluorometers, and spectrophotometers.
3. Pipette 100 μl of the Firefly Lantern Extract Solution prepared in step 1 into an appropriate tube or cuvette.
4. Place tube in instrument and record the intensity of the background light emitted.
5. Pipette 20 μl of the sample preparation into the tube. Mix gently and immediately start timer.
6. After 20 seconds measure and record the intensity of the emitted light.
   Notes: Peak wavelength emission is reported at 560 nm when the reaction is conducted at pH 7.8.[5] A lower pH, such as 6.0, will cause a shift in maximum intensity to 620 nm.

   Duration of the Light Flash - Light emission peaks in about one second and then decays rapidly with a typical half-life of one minute. Arsenate will extend the half-life substantially, but with diminished sensitivity for the detection of ATP. In general, the intensity of light emission may be conveniently recorded after a 20-second time period. If automated sample injection capacity is available, a three-second time period may be appropriate.

7. Subtract the background light measured in step 4 from the emitted light measured in step 6 to obtain a corrected value.
8. Repeat steps 3–7 substituting the ATP Standard Solutions in place of the sample.
9. Calculate the ATP concentration of the sample by comparing the corrected value of the sample to those of the ATP Standards. An ATP standard curve generated from 3 ATP Standard Solutions of different concentrations will generally suffice.

References

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5. Nilsson, L., et al., Rapid detection of bacteriemia by bioluminescent assay of bacterial ATP. Anal. Appl. Biolumin. Chemilumin. (Proc. Int. Symp.), 3rd, LJ Kricka, Editor, Academic Press, London (1984), pp 25-28.
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