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Product Information

Adenosine 5′-triphosphate (ATP)
Bioluminescent Assay Kit

Catalog Number **FLAA**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Adenosine 5′-triphosphate (ATP) Bioluminescent Assay Kit may be employed for the quantitative bioluminescent determination of Adenosine 5′-triphosphate (ATP) in samples containing 2×10^{-12} to 8×10^{-5} moles/liter (or 10^{-12} to 4×10^{-5} grams/ml based on a MW of 507 g/mole for the free acid). ATP is consumed and light is emitted when luciferase catalyzes the oxidation of D-luciferin:

$$\text{ATP + Luciferin} \underset{\text{Mg}^{++}}{\overset{\text{Luciferase}}{\rightleftharpoons}} \text{Adenyl-luciferin + PP}_i \quad (1)$$

$$\text{Adenyl-luciferin + O}_2 \rightarrow \text{Oxyluciferin + AMP + CO}_2\text{+ light} \quad (2)$$

Reaction (1) is reversible and the equilibrium lies far to the right. Reaction (2) is essentially irreversible.[1,2] When ATP is the limiting reagent, the light emitted is proportional to the ATP present.

Components

ATP Assay Mix
lyophilized powder containing luciferase, luciferin, $MgSO_4$, DTT, EDTA, BSA, and tricine buffer salts.
(Catalog Number FLAAM)

ATP Assay Mix Dilution Buffer
lyophilized powder containing $MgSO_4$, DTT, EDTA, bovine serum albumin, and tricine buffer salts.
(Catalog Number FLAAB)

ATP Standard
preweighed vial contains ~1 mg (2.0×10^{-6} mole) of adenosine 5′-triphosphate. Actual ATP content is given on product label.
(Catalog Number FLAAS)

Precautions and Disclaimer

This kit is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

ATP Assay Mix, (Stock Solution) – The contents of one vial of ATP Assay Mix (Catalog Number FLAAM) should be dissolved in 5 ml of sterile water to generate a stock solution with pH of 7.8. Mix by **gentle** inversion or swirling until dissolved. Allow the solution to stand in ice for at least one hour to assure complete dissolution. During this time, a decrease in background may also be seen. This solution is stable for at least two weeks when stored at 0–5 °C and protected from light. A slight decrease in light production and sensitivity may occur during this time. **Therefore, a new standard curve must be prepared each day before use.** The stock solution may be dispensed in aliquots and frozen for future use. It is also stable for 2–3 freeze-thaw cycles.

The ATP Assay Mix stock solution as prepared above is effective for determining the ATP concentrations in samples ranging from 2×10^{-12} to 2×10^{-9} moles/liter. If a less sensitive system is necessary, simply dilute the ATP Assay Mix stock solution with ATP Assay Mix Dilution Buffer. A 25-fold dilution of ATP Assay Mix stock solution is suitable for detecting sample ATP concentrations in the range of 2×10^{-10} to 2×10^{-7} moles/liter, and a 625-fold dilution is suitable for a range of 8×10^{-8} to 8×10^{-5} moles/liter (see Figure 1). The appropriate dilution of ATP Assay Mix stock solution will depend upon the amount of ATP present in the "unknown" sample and the specific instrument used.

ATP Assay Mix Dilution Buffer, (Stock Solution) – The contents of one vial of ATP Assay Mix Dilution Buffer (Catalog Number FLAAB) should be dissolved in 50 ml of sterile water. This solution is stable for at least two weeks at 0–5 °C.

ATP Standard, (Stock Solution) – An ATP Standard stock solution may be prepared by dissolving the contents of one vial of ATP Standard (Catalog Number FLAAS) with sterile water. This solution is stable for at least 24 hours at 2–8 °C, or over two weeks if stored frozen at –20 °C. Prepare the ATP Standard solutions by making serial dilutions of ATP Standard stock solution with sterile water. The extent of dilution depends upon the assay sensitivity desired. These dilutions are stable for up to 8 hours when stored in ice.

Storage/Stability

ATP Assay Mix is stable indefinitely if stored desiccated at –20 °C and protected from light.

ATP Assay Mix Dilution Buffer is stable indefinitely if stored desiccated at –20 °C.

ATP Standard should be stored desiccated at –20 °C.

Procedure

Note: In preparing a sample for assay, it is important that the pH be adjusted to ~7.8. Sterile distilled water is recommended if dilution of the sample solution is required, or for dissolving a solid sample. It is advised not to use arsenate as the sample buffer, since it tends to lower the sensitivity through quenching.[4] Also, high salt concentrations in the sample will have a general inhibitory effect on the luciferase and will decrease sensitivity.[5,6] The K_M for ATP increases with increasing ionic strength.

1. Add 0.1 ml of ATP Assay Mix solution to a reaction vial. Swirl and allow to stand at room temperature for ~3 minutes. During this period, any endogenous ATP will be hydrolyzed, thereby decreasing the background. A series of up to 15 assays may be set up at one time.

2. Begin the assay by rapidly adding 0.1 ml of standard or sample. Swirl briskly to mix and immediately measure the amount of light produced with a luminometer.

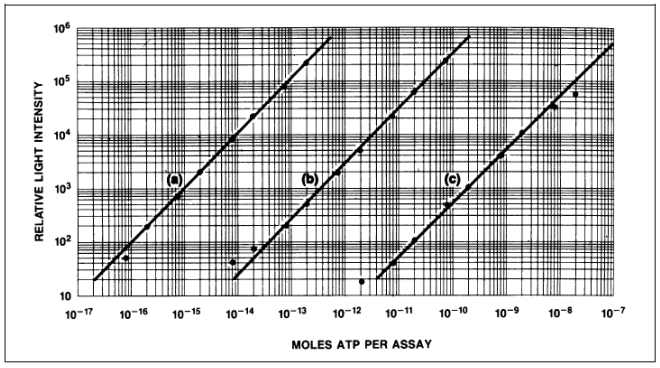
3. It is important to determine the amount of background light produced by running a blank. Add 0.1 ml of ATP Assay Mix solution to a reaction vial and allow to stand at room temperature for ~3 minutes. Then rapidly add 0.1 ml of sterile water (or sample diluent), swirl briskly to mix, and immediately measure the amount of light produced. Subtract this value from that obtained for the sample. This final value is proportional to the amount of ATP in the sample.

4. A blank for the ATP Standards may be determined in a similar manner. Add 0.1 ml of ATP Assay Mix solution to a reaction vial and allow to stand at room temperature for ~3 minutes. Rapidly add 0.1 ml of sterile water, swirl briskly to mix, and immediately measure the amount of light produced. Subtract this value from that obtained for each standard. These final values are proportional to the amount of ATP in the standards.

   See the typical calibration curves (see Figure 1).

   Note: The typical calibration curve depicted below cannot be used to derive laboratory test results. Each laboratory must prepare its own calibration curve.

Figure 1.
Typical ATP Calibration Curves



The assays were performed in 15 mm × 60 mm glass vials. The instrument was preset to integrate the amount of light produced over a 6-second interval without an initial delay. The ATP Assay Mix stock solution was (a) undiluted, (b) diluted 25-fold and (c) diluted 625-fold.

References

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TAD,GCY,JWM,MAM 12/15-1