ATP Determination Kit (A22066)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light

Number of assays: 200–1000

Introduction

Molecular Probes' ATP Determination Kit (A22066) offers a convenient bioluminescence assay for quantitative determination of ATP with recombinant firefly luciferase and its substrate D-luciferin (Figure 1). The assay is based on luciferase's requirement for ATP in producing light (emission maximum ~560 nm at pH 7.8) from the reaction:

$$\text{luciferin} + \text{ATP} + O_2 \xrightarrow[\text{luciferase}]{Mg^{2+}} \text{oxyluciferin} + \text{AMP} + \text{pyrophosphate} + CO_2 + \text{light}$$

This assay is extremely sensitive; most luminometers can detect as little as 0.1 picomole of pre-existing ATP, or ATP as it is being formed in kinetic systems.[1] This sensitivity has led to numerous applications for detecting ATP production in various enzymatic reactions, including ATPase [2] and NADPH oxidase,[3] as well as for detecting low-level bacterial contamination in samples such as blood, milk, urine, soil and sludge.[4-8] The luciferin–luciferase bioluminescence assay has also been used successfully to study the effects of antibiotics on bacterial populations [9] and to distinguish cytostatic versus cytocidal potential of anticancer drugs on malignant cell growth.[10]

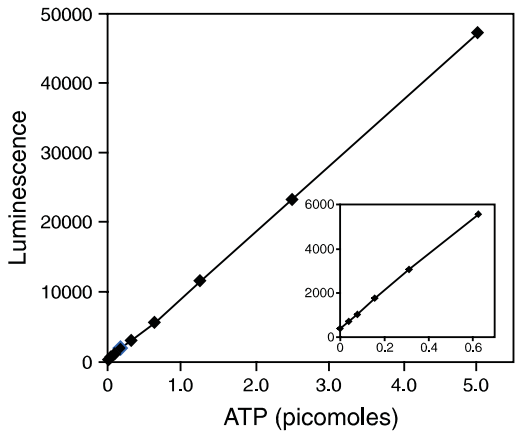

Figure 1. Detection of ATP using the ATP Determination Kit. Each reaction contained 1.25 µg/mL of firefly luciferase, 50 µM D-luciferin and 1 mM DTT in 1X Reaction Buffer. After a 15 minute incubation, luminescence was measured (arbitrary units).

Molecular Probes' ATP Determination Kit provides all of the reagents needed to perform this luminescence assay. In this kit, D-luciferin and firefly luciferase are packaged separately rather than premixed. The separate packaging allows researchers to optimize the proportions of D-luciferin and firefly luciferase for their particular system. This flexibility can be useful because the detection range of ATP will vary depending on instrument and sample requirements. A standard protocol is included that should be suitable for many routine applications; this protocol should also provide a starting point for customizing the assay. In addition to firefly luciferase and D-luciferin, this kit supplies a 20X concentrate of reaction buffer, dithiothreitol (DTT) for use in the reaction and an ATP solution for preparing standard curves.

Materials

Kit Contents

- **D-Luciferin** (Component A, MW 302, blue cap), 5 vials, each containing 3 mg of lyophilized powder
- **Luciferase, firefly recombinant** (Component B, red cap) 40 µL of a 5 mg/mL solution in 25 mM Tris-acetate, pH 7.8, 0.2 M ammonium sulfate, 15% (v/v) glycerol and 30% (v/v) ethylene glycol
- **Dithiothreitol (DTT)** (Component C, MW 154, black cap) 25 mg
- **Adenosine 5′-triphosphate (ATP)** (Component D, green cap), 400 µL of a 5 mM solution in TE buffer
- **20X Reaction Buffer** (Component E) 10 mL of 500 mM Tricine buffer, pH 7.8, 100 mM $MgSO_4$, 2 mM EDTA and 2 mM sodium azide

The ATP Determination Kit provides sufficient reagents to perform 200 ATP assays using 500 µL sample volumes or 1000 ATP assays using 100 µL sample volumes. The contents of this kit are sufficient to make at least 100 mL of a standard reaction solution (see step 2.1) containing 0.5 mM D-luciferin, 1.25 µg/mL firefly luciferase, 25 mM Tricine buffer, pH 7.8, 5 mM $MgSO_4$, 100 µM EDTA and 1 mM DTT.

Storage And Handling

Upon receipt, this kit should be stored at ≤–20°C, protected from light. For long term storage and infrequent use, store the

firefly luciferase frozen at –80°C. Avoid repeated freezing (at –80°C) and thawing. When properly stored, these reagents are stable for six months to one year.

Experimental Protocol

The following protocol has been developed as a generalized procedure suitable for many experimental applications. Optimal reaction conditions for specific experimental circumstances may be determined empirically by adjusting the amounts of D-luciferin and firefly luciferase added to the reaction. In the protocol below, a 10 mL "standard reaction solution" is prepared. This volume of standard reaction solution is sufficient for about twenty 500 µL reactions or about one hundred 100 µL reactions, depending upon luminometer requirements. For fewer or more assays, modify the protocol accordingly.

Special Precautions

- Because of the high sensitivity of the luciferin–luciferase reaction, avoid contamination with ATP from exogenous biological sources, such as bacteria or fingerprints.
- Protect the D-luciferin and firefly luciferase reagents from light as much as possible.
- Mix solutions containing firefly luciferase *gently*, for example, by inversion; vortex mixing may denature the enzyme.
- Arsenate compounds may inhibit the reaction.
- The optimum temperature for the reaction is 28°C. At higher temperatures, the reaction is slower.

Reagent Preparation

1.1 Make 1.0 mL of 1X Reaction Buffer by adding 50 µL of 20X Reaction Buffer (Component E) to 950 µL of deionized water (dH_2O). This volume will be sufficient to make 1 mL of 10 mM D-luciferin stock solution.

1.2 Make 1 mL of a 10 mM D-luciferin stock solution by adding 1 mL of 1X Reaction Buffer (prepared in step 1.1) to one vial of D-luciferin (Component A, blue cap). Protect from light until use. The D-luciferin stock solution is reasonably stable for several weeks if stored at ≤–20°C, protected from light.

1.3 Prepare a 100 mM DTT stock solution by adding 1.62 mL of dH_2O to the bottle containing 25 mg of DTT (Component C, black cap). Aliquot into ten 160 µL volumes and store frozen at ≤–20°C. Stock solutions of DTT stored properly are stable for six months to one year. Thawed aliquots should be kept on ice or at 4°C until ready for use.

1.4 Prepare low-concentration ATP standard solutions by diluting the 5 mM ATP solution (Component D, green cap) in dH_2O. The concentrations and volumes to make depend upon the sensitivity and design of the luminometer to be used. Typically, ATP concentrations ranging from 1 nM to 1 µM are appropriate. These dilute solutions are stable for several weeks when stored at ≤–20°C.

Standard Reaction Solution

2.1 We suggest combining the components of the reaction as follows to make 10 mL of a standard reaction solution. Adjust the volumes according to particular requirements.

- 8.9 mL dH_2O
- 0.5 mL 20X Reaction Buffer (Component E)
- 0.1 mL 0.1 M DTT (from step 1.3)
- 0.5 mL of 10 mM D-luciferin (from step 1.2, store the remaining 0.5 mL at ≤–20°C for up to several weeks)
- 2.5 µL of firefly luciferase 5 mg/mL stock solution

2.2 Gently invert the tube to mix, **do not vortex;** the firefly luciferase enzyme is easily denatured. Keep the reaction solution protected from light until use. Although the solution may be stored at 2–6°C protected from light for several days, assay sensitivity will diminish with time (see *Notes*).

Standard Curve

3.1 Place an appropriate volume of the standard reaction solution (prepared in steps 2.1 and 2.2) in the luminometer and measure the background luminescence.

3.2 Start the reaction by adding the desired amount of dilute ATP standard solution (prepared in step 1.4) and read the luminescence. The volume of the dilute ATP standard solution that is added to the standard assay solution (prepared in step 2.1) should be no more than 10% of the total assay volume. For example, a 100 µL total assay volume should contain 10 µL or less of the ATP standard solution.

3.3 Subtract the background luminescence.

3.4 Generate a standard curve for a series of ATP concentrations. Be sure to always add a constant sample volume of the ATP-containing solution.

Sample Analysis

4.1 Follow the directions given in *Standard Curve*, substituting ATP-containing samples for the ATP standard solutions. Please note that the total volume of the experimental sample assays should be equal to that of the ATP standard assays, with the amount of sample added amounting to no more than 10% of the total assay volume.

4.2 Calculate the amount of ATP in the experimental samples from the standard curve.

Notes

Standards must be run with each experiment. Fluorescence background levels of the working solutions increase over time, decreasing the sensitivity of the assay.

References

1. J Appl Biochem 3, 473 (1981); **2.** Biotechniques 31, 420 (2001); **3.** J Biol Chem 276, 3090 (2001); **4.** Anal Biochem 175, 14 (1988); **5.** Bio/Technology 6, 634 (1988); **6.** J Clin Microbiol 20, 644 (1984); **7.** J Clin Microbiol 18, 521 (1983); **8.** Meth Enzymol 57, 3 (1978); **9.** J Biolumin Chemilumin 6, 193 (1991); **10.** J Natl Cancer Inst 77, 1039 (1986).

Product List *Current prices may be obtained from our website or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A22066	ATP Determination Kit *special packaging* *200-1000 assays*	1 kit

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