TECHNICAL BULLETIN

# ENLITEN® ATP Assay System Bioluminescence Detection Kit for ATP Measurement

Instructions for Use of Product
**FF2000**


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# ENLITEN® ATP Assay System Bioluminescence Detection Kit for ATP Measurement

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**E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com**



## 1. Description

The ENLITEN® ATP Assay System is intended for the rapid and quantitative detection of adenosine 5´-triphosphate (ATP). The rL/L Reagent included in the system is designed to measure $10^{-11}$ to $10^{-16}$ moles of ATP.

Some of the many applications for the ENLITEN® ATP Assay System include:

- Indirect measurement of bacteria, yeasts, fungi and other microorganisms in foodstuffs, beverages, water, woodpulp, cosmetics and other products.
- Assay of enzymes that produce or degrade ATP.

The assay uses recombinant luciferase to catalyze the following reaction (1):

ATP + D-Luciferin + $O_2$ → Oxyluciferin + AMP + PPi + $CO_2$ + Light (560nm)

When ATP is the limiting component in the luciferase reaction, the intensity of the emitted light is proportional to ATP concentration. Measurement of the light intensity using a luminometer permits direct quantitation of ATP (2,3).

## 1. Description (continued)

The ATP Assay System can be used to measure microorganisms and cells by first treating them with a permeabilizing or lysis reagent to release ATP. Tissues require homogenization to release cells before permeabilizing/lysis.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **ENLITEN® ATP Assay System** | **100 assays** | **FF2000** |

Components include:

- 1 vial rL/L Reagent
- 12ml Reconstitution Buffer
- 1 vial ATP Standard ($10^{-7}$M)
- 25ml ATP-Free Water

**Storage Conditions:** Prior to reconstitution, the rL/L Reagent and Reconstitution Buffer must be stored at –20°C. Store the ATP Standard at –20°C.

Wear new, disposable gloves when handling the rL/L Reagent.

## 3. Reconstitution of rL/L Reagent

Use only clean, disposable pipettes to reconstitute the rL/L Reagent. It is very important to use clean equipment and wear gloves to prevent contamination by trace amounts of ATP present in fingerprints, glassware, etc. Do not touch the outside of the gloves with your fingers or skin.

Before opening, gently tap the rL/L Reagent vial to insure that the lyophilized material is in the vial bottom. Slowly remove the vial crimp seal and rubber stopper to avoid loss of material. Add the entire contents of the plastic bottle labeled Reconstitution Buffer to the vial, replace the rubber stopper and gently swirl the vial to dissolve the contents. **DO NOT** shake the dissolved rL/L Reagent. Allow the rL/L Reagent to incubate at room temperature for 1 hour before using.

Reconstituted rL/L Reagent can be held at room temperature for 8 hours. If the reagent will be used for longer than 8 hours, dispense the rL/L Reagent into aliquots, and store them at 4°C, protected from light. Remove aliquots as needed. The activity of the reconstituted rL/L Reagent diminishes roughly 15% after 2 days of storage at 4°C. Be sure to allow the rL/L Reagent to return to room temperature prior to use.

If long-term storage is needed, the reconstituted rL/L Reagent can be stored in single-use aliquots at –20°C. Avoid multiple freeze-thaws. The activity of reconstituted rL/L Reagent diminishes by roughly 50% after two weeks at –20°C.

## 4. ATP Assay Procedure

### 4.A. Sample Preparation

There are many possible assay schemes for ATP analysis using firefly luciferase bioluminescence (4–9). Sample preparation is highly important and depends on what ATP-containing component is to be measured and the physical properties of the sample. The sample preparation procedure should minimize inhibition of rL/L light output by sample components or assay conditions. The rL/L reaction is optimum at pH 7.75 and 23–25°C and is inhibited by salts and many nonionic chemicals even at low concentrations. Buffers and ATP extractants used in sample preparation should be checked to make sure they are free of contaminating ATP and substances that may inhibit the rL/L reaction. To check for ATP contamination, use equal volumes of the component to be tested and the rL/L Reagent (for example, 0.1ml of each). Using the same amount of ATP in each reaction, the relative light units (RLU) with buffer/extracts should not be significantly different from the RLU with ATP-Free Water. If the buffers/extracts are found to contain ATP or inhibit the rL/L reaction, this must be compensated for in calculating the amount of ATP present in the sample (see Section 4.D).

**!** Wear new, disposable gloves when preparing samples and performing the ATP Assay.

It is important to design your ATP measurement procedure to insure that the proper ATP pool is measured. Often, biological samples contain many different types of cells as well as ATP free in solution. If a certain type of cell is to be quantitated, such as bacterial cells contaminating a foodstuff, those cells must first be isolated before lysis and ATP measurement. Many isolation schemes are possible, including centrifugation, filtration, chromatography and precipitation. Also, if accurate cell quantitation is desired, ATP in a known number of isolated cells should be measured to establish the average amount of ATP per cell for future reference. To obtain an accurate average ATP content per cell, the isolated cells should be treated the same as the cells isolated from samples, since environmental factors such as pH, light, pressure due to centrifugation or filtration, buffer chemicals, etc., may affect intracellular ATP levels (10).

### 4.B. ATP Extraction

If ATP in microorganisms or cells is to be measured, it must be extracted efficiently without degradation. A wide variety of ATP-extracting reagents have been described (10,11). Generally, the best extractant is trichloroacetic acid (TCA). TCA efficiently releases ATP from microorganisms and cells while inactivating enzymes that might quickly degrade the ATP before measurement. Usually 0.5% to 2.5% TCA (final concentration) in a sample will extract ATP from bacteria and eukaryotic cells. As much as 5% TCA may be required to extract ATP from yeasts, fungi, and algae. Because TCA inhibits the rL/L reaction, the lowest concentration of TCA needed for extraction should be used. It is convenient to include a small amount (0.0005% to 0.002%) of xylenol blue dye in the TCA.

### 4.B. ATP Extraction (continued)

The dye should turn red after adding TCA to the sample, indicating pH <1.2. After TCA extraction, the TCA in the sample should be neutralized and diluted to a final concentration of 0.1% or less by adding a pH 7.75 buffer, such as Tris-acetate (12,13). After adding the neutralizing buffer, the dye should turn yellow. The solution pH should be checked and adjusted if necessary to pH 7.75 with additional buffer.

### 4.C. Luminometer Preparation

Light output from the rL/L reaction is measured in either a tube-reading or microwell plate-reading luminometer. If performing assays in multiwell plates, use opaque-walled plates to minimize cross-talk between wells. The luciferase reaction is very rapid and begins immediately upon addition of reagent to the sample, resulting in a flash of light. Therefore, it is recommended to process samples individually by adding reagent and immediately recording light output. A luminometer with a reagent delivery system is recommended for the best consistency between the timing of reagent addition and measuring light output. Refer to the instruction manual of your luminometer for proper instrument setup and operation. If your luminometer has a reagent delivery system, it is important that the reagent injectors and supply tubing are kept clean and aseptic. A 2% bleach (sodium hypochlorite) solution can usually be used to remove bacteria and traces of ATP from the reagent injector and tubing (check your instrument manual or contact the manufacturer). To do this, prime the reagent injector with the bleach solution, and allow it to sit for 1 hour. Rinse the injector by priming 10 times with sterile, distilled or deionized water. Be sure to carefully rinse any filters on the reagent tubing as well. Proper care of your luminometer is important for low assay "background" and precision in ATP measurements.

### 4.D. Performing the ATP Assay

Once reconstituted, the rL/L Reagent is sufficient for 100 ATP assays, using 100µl of reagent with 100µl of sample for each assay. Refer to your luminometer instrument manual for the recommended procedure for ATP assays. The ENLITEN® ATP Assay System has been designed for use in most luminometer protocols. Use disposable pipette tips when adding rL/L Reagent, sample, buffers/water, extractant or ATP Standard; use a new tip for each addition. Pipet gently to avoid generating aerosols that could contaminate assay reagents.

A "blank", containing rL/L Reagent and sample buffer/extractant, should be run in the assay to determine the amount of "background" RLU that will be subtracted from the sample RLU. Sample RLU values should be corrected for possible buffer/extractant inhibition of light output when converting RLU to ATP mass. This is done by constructing an ATP standard curve (see Figure 1) using an appropriate volume of buffer/extractant instead of water to dilute the ATP Standard. It is recommended that samples and ATP Standard curve are done in at least duplicate for accurate measurement.

**Note:** A new ATP Standard curve must be made fresh daily, or whenever a new aliquot of the rL/L Reagent is used. The coefficient of variation (Standard Deviation divided by the average RLU) for each set of measurements should be 10% (0.10) or less.

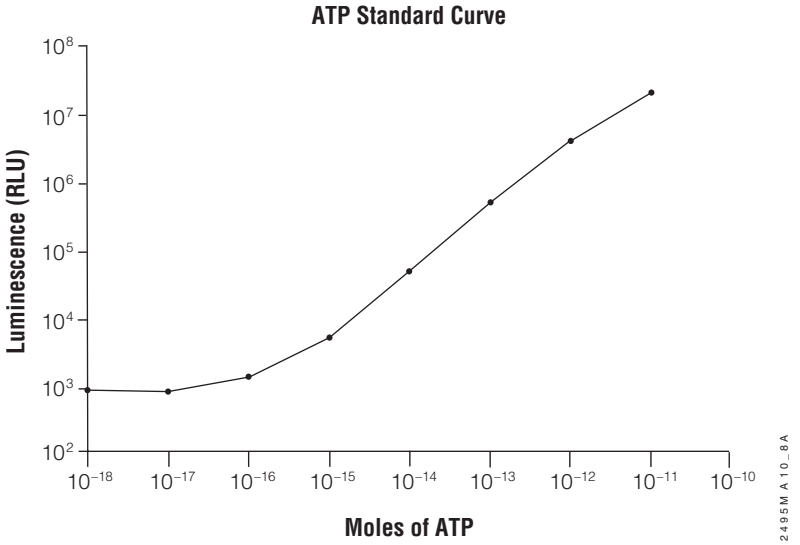


**Figure 1. A representative ATP standard curve obtained using the ENLITEN® ATP Assay System.** Ten microliters of ATP Standard diluted to the proper concentrations was added to a cuvette and assayed using rL/L Reagent according to the luminometer protocol. A 2-second delay time after L/L Reagent injection and a 10-second RLU signal integration time were used.

**Note:** This ATP standard curve is for **illustration only**. It is important to make a standard curve for each ATP assay system using your luminometer and sample buffer.

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## 5. References


1. DeLuca, M.A and McElroy, W.D. (1978) Purification and properties of firefly luciferase. *Methods Enzymol.* **57**, 3–15.
2. McElroy, W.D. and Deluca, M.A. (1983) Firefly and bacterial luminescence: Basic science and applications. *J. Appl. Biochem.* **5**, 197–209.
3. Lundin, A. and Thore, A. (1975) Analytical information obtainable by evaluation of the time course of firefly bioluminescence in the assay of ATP. *Anal. Biochem.* **66**, 47–63.
4. DeLuca, M.A. (1976) Firefly luciferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **44**, 37–68.
5. Lundin, A., Richardsson, A. and Thore, A. (1976) Continuous monitoring of ATP-converting reactions by purified firefly luciferase. *Anal. Biochem.* **75**, 611–20.
6. Webster, J.J. and Leach, F. (1980) Optimization of the firefly luciferase assay for ATP. *J. Appl. Biochem.* **2**, 469–79.
7. Lundin, A. (1982) In: *Luminescent Assays: Perspectives in Endocrinology and Clinical Chemistry*, M. Serio and M. Pazzagti, eds. Raven Press, New York.
8. Kricka, L.J. (1988) Clinical and biochemical applications of luciferases and luciferins. *Anal. Biochem.* **175**, 14–21.
9. Gould, S.J. and Subramani, S. (1988) Firefly luciferase as a tool in molecular and cell biology. *Anal. Biochem.* **175**, 5–13.
10. Karl, D.M. (1980) Cellular nucleotide measurements and applications in microbial ecology. *Microbiol. Rev.* **44**, 739–96.
11. Stanley, P.E. (1986) Extraction of adenosine triphosphate from microbial and somatic cells. *Methods Enzymol.* **133**, 14–22.
12. Webster, J.J. *et al.* (1980) Buffer effects on ATP analysis by firefly luciferase. *Anal. Biochem.* **106**, 7–11.
13. Nichols, W.W., Curtis, G.D. and Johnson, H.H. (1981) Choice of buffer anion for the assay of adenosine 5´-triphosphate using firefly luciferase. *Anal. Biochem.* **114**, 396–7.

## 6. Related Products

| Product | Size | Cat.# |
|---|---|---|
| ENLITEN® rLuciferase/Luciferin Reagent | 100 assays | FF2021 |
| BacTiter-Glo™ Microbial Cell Viability Assay | 10ml | G8230 |
| | 10 × 10ml | G8231 |
| | 100ml | G8232 |
| | 10 × 100ml | G8233 |
| CellTiter-Glo® Luminescent Cell Viability Assay | 10ml | G7570 |
| CellTiter-Glo® 2.0 Assay | 10ml | G9241 |
| CellTiter-Glo® 3D Cell Viability Assay | 10ml | G9681 |
| GloMax® 20/20 Luminometer | 1 each | E5311 |
| GloMax® 20/20 Luminometer with Single Auto-Injector | 1 each | E5321 |
| GloMax® 20/20 Luminometer with Dual Auto-Injector | 1 each | E5331 |
| GloMax® Discover System | 1 each | GM3000 |
| GloMax® Navigator System | 1 each | GM2000 |

## 7. Summary of Changes

The following changes were made to the 9/17 revision of this document:

1. An error regarding bleach concentration was corrected in Section 4.C.
2. New products were added to Related Products.