TECHNICAL BULLETIN

ENLITEN® rLuciferase/ Luciferin Reagent

A Bioluminescence Detection Reagent for ATP Measurement

Instructions for Use of Product
FF2021

Revised 11/15
TB268

ENLITEN® rLuciferase/ Luciferin Reagent

All technical literature is available at: www.promega.com/protocols/
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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® rLuciferase/Luciferin (rL/L) Reagent is intended for the rapid and quantitative detection of adenosine 5´-triphosphate (ATP). The rL/L Reagent is designed to measure 10^{-11} to 10^{-16} mole ATP.

Some of the many applications for the ENLITEN® rL/L Reagent 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.
- Quantitation of ATP in biological fluids.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ENLITEN® rLuciferase/Luciferin Reagent	**100 assays**	**FF2021**

Components include:

- 1 vial rLuciferase/Luciferin Reagent
- 12ml Reconstitution Buffer

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

3. Principle of the Assay

Recombinant luciferase catalyzes the following reaction (1):

ATP + D-Luciferin + O_2 → Oxyluciferin + AMP + Pyrophosphate + 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).

4. Reagent Composition and Testing

4.A. rLuciferase/Luciferin (rL/L) Reagent

The rL/L Reagent is supplied lyophilized; once it has been reconstituted with Reconstitution Buffer, it contains purified luciferase, D-luciferin, Tris-acetate buffer (pH 7.75), ethylenediaminetetraacetic acid (EDTA), magnesium acetate, bovine serum albumin (BSA) and dithiothreitol (DTT). Sodium azide (0.02%) is included as a preservative.

4.B. Reconstitution Buffer

The Reconstitution Buffer contains buffer, salts and other components that must be added to the vial of lyophilized rL/L Reagent to reconstitute the reagent.

5. rL/L Reagent Reconstitution

Wear new, disposable gloves when handling 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 ensure that the lyophilized material is in the bottom of the vial. 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, recap with 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 rehydrate 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 the reconstituted rL/L Reagent diminishes by roughly 50% after two weeks at –20°C.

6. ATP Assay Procedure

Wear new, disposable gloves when preparing samples and performing the ATP assay.

6.A. Luminometer Preparation

Light output from the L/L reaction is usually measured in a luminometer. Refer to the instruction manual of your luminometer for proper instrument setup and operation. Proper care of your luminometer is important for low assay "background" and precision in ATP measurements. If your instrument has a reagent delivery system, it is essential that the reagent injectors and supply tubing are kept clean and aseptic. If the instrument manual does not contain instructions for the cleaning and maintenance of the injectors, contact the manufacturer for instructions. After cleaning the injectors, rinse the system by priming 10 times with sterile, distilled or deionized water. Be sure to carefully rinse any filters on the reagent tubing as well.

6.B. Performing the ATP Assay

Once reconstituted, the rL/L Reagent is sufficient for 100 ATP assays, assuming 0.1ml reagent per assay. Refer to your luminometer instrument manual for the recommended procedure for ATP assays. The rLuciferase/ Luciferin Reagent has been designed for use in most luminometer protocols. Use disposable pipette tips when adding rL/L Reagent, sample, buffer/water, extractant or ATP standard; use a new tip for each addition. Pipet gently to avoid generating aerosols that could contaminate assay reagents.

6.B. Performing the ATP Assay (continued)

A "blank" containing rL/L Reagent and the sample buffer and extractant used to prepare your samples should be run in the assay to determine the amount of "background" relative light units (RLU) to 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 (Figure 1) using an appropriate volume of buffer/extractant instead of water to dilute the ATP standard. We recommend that samples and the ATP standard curve are performed in at least duplicate for accurate measurement (3). The percent coefficient of variation (standard deviation divided by the mean RLU times 100) for each set of measurements should be 10% or less over the entire assay range.

Note: A new ATP standard curve must be made fresh daily or whenever a new aliquot of rL/L Reagent is used.

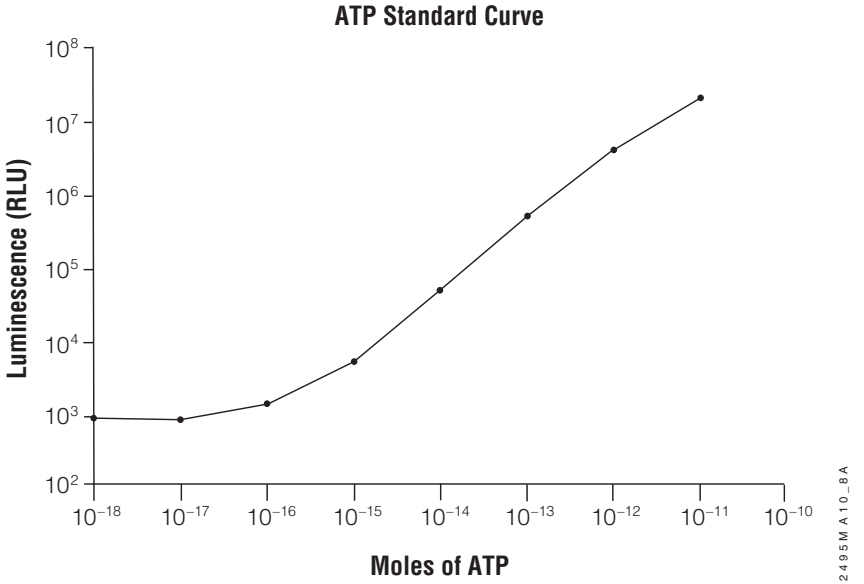

Figure 1. A representative ATP standard curve obtained using the ENLITEN® rL/L Reagent and a Berthold Luminometer Model 9501. Ten microliters of ATP standard diluted to the proper concentrations was added to a cuvette and assayed using rL/L Reagent according to the luminometer protocol. A 1-second delay time after rL/L Reagent injection and 10-second RLU signal integration time were used.

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

7. 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.

8. Related Products

Product	Size	Cat.#
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® 96 Microplate Luminometer	1 each	E6501
GloMax® 96 Microplate Luminometer with Single Injector	1 each	E6511
GloMax® 96 Microplate Luminometer with Dual Injectors	1 each	E6521

9. Summary of Changes

The following changes were made to the 11/15 revision of this document:

1. The patent information was updated to remove expired statements.
2. The document design was updated.