

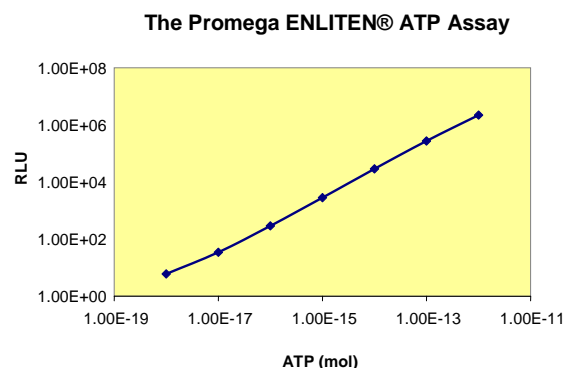
# A GloMax<sup>®</sup> Multi Microplate Luminometer Method for the ENLITEN<sup>®</sup> ATP Assay

## INTRODUCTION

The GloMax<sup>®</sup> Multi Microplate Luminometer combined with the ENLITEN<sup>®</sup> ATP Assay System (Cat.# FF2000) provides a highly sensitive, quantitative method for rapidly measuring adenosine 5'-triphosphate (ATP). Some of the many applications for the ENLITEN<sup>®</sup> ATP Assay System include indirect measurement of bacteria, yeasts, fungi and other microorganisms and assay of enzymes that produce or degrade ATP.

The ATP-dependent oxidation of luciferin by luciferase produces light measured by the GloMax<sup>®</sup> Multi Microplate Luminometer. When ATP is the limiting factor in the luciferin oxidation reaction, the amount of light produced is proportional to the ATP concentration of the sample.

The highly sensitive GloMax<sup>®</sup> Multi Microplate Luminometer with injector can detect attomole levels of ATP. The limit of detection for ATP with the ENLITEN<sup>®</sup> Assay is 1 attomoles or 0.5 femtograms.



**Figure 1.** A standard curve is obtained using the GloMax<sup>®</sup> Multi Microplate Luminometer and the ENLITEN<sup>®</sup> ATP Assay System. 100  $\mu$ L rL/L Reagent was injected into sample wells of a 96-well plate containing 10  $\mu$ L of ATP Standard diluted in HEPES buffer.

When designing an ATP assay with the ENLITEN<sup>®</sup> System, it is important to remember several key aspects of the luciferase reaction.

First, rL/L reacts optimally at 7.73 pH and 23–25°C. Salts and many nonionic chemicals will impair light production. Therefore, exercise caution when selecting buffers and ATP extractants for the sample preparations. Second, check for ATP contamination by comparing the RLU values obtained from the assay buffer and rL/L to those of ATP-Free Water.

There may exist several different ATP stores within any given sample. For example, in cell preparations, ATP may be present in the media. Treatment of cells may alter the amount of ATP in the media.

To measure the ATP content in microorganisms or cells, extract the ATP before analysis. Trichloroacetic acid (TCA) is recommended to release ATP from cells and inactivate ATP-degrading enzymes.

Determining the minimum amount of TCA is crucial because TCA inhibits the luciferase reaction. A final concentration of 0.5 –2.5% TCA is sufficient for ATP extraction from bacteria and eukaryotic cells.

Standard curves are useful tools for proper ATP analysis of samples. Prepare a standard curve daily or whenever a new aliquot of the rL/L Reagent is used. The standard curve should include varying concentrations of ATP diluted in assay buffer. The composition of this buffer should be identical to the buffer composition used in assay sample preparation. Minding these considerations safeguard the highest possible accuracy of ATP analysis.

## MATERIALS REQUIRED

- GloMax<sup>®</sup> Multi Microplate Multimode Reader
- GloMax<sup>®</sup> Multi Microplate Luminescence Module

- GloMax<sup>®</sup> Multi Microplate Injector System
  - ENLITEN<sup>®</sup> ATP Assay System Bioluminescence Detection Kit for ATP (Cat.# FF2000) containing one vial rLuciferase/Luciferin (rL/L) Reagent, 12 mL Reconstitution Buffer, one vial ATP Standard ( $1 \times 10^{-7}$  M), 25 mL ATP-Free Water
  - 96-well, white microplates (E&K Scientific, Cat.# EK-25075)
  - P10, P200 pipette and pipette tips
  - assay buffer
  - 1.5 mL microfuge tubes
  - nitrile, vinyl, or latex gloves
- Note:** Individuals sensitive to latex should use vinyl or nitrile gloves.

**Storage Conditions:** The rL/L Reagent and Reconstitution Buffer must be stored at  $-20^{\circ}\text{C}$  prior to reconstitution. Experimental protocol

## EXPERIMENTAL PROTOCOL

### Reagent and Standard Preparation

**Note:** ATP contamination will cause erroneous results and increase background. Wear gloves to prevent hand-transmitted ATP contamination during reagent preparation and while performing the assay

1. Equilibrate the sample buffer to room temperature.
2. Lightly tap the vial of rL/L Reagent before opening to ensure the lyophilized material collects at the bottom of the vial.
3. Transfer the vial contents of rL/L Reconstitution Buffer into the vial of rL/L Reagent.
4. Replace the stopper and slowly invert the vial several times to dissolve the contents. Do not shake the reagent bottle.
5. Allow the reconstituted rL/L Reagent to stand at room temperature for one hour before use.
6. **Note:** Reconstituted rL/L Reagent may be kept for eight hours at room temperature. Store at  $-20^{\circ}\text{C}$  in single-use aliquots for long-term storage.

7. Prepare a 10-fold serial dilution of ATP Standard ( $1 \times 10^{-7}$  M) in assay buffer. Dilute ATP to  $1 \times 10^{-13}$  M.

### Instrument Set Up

1. For ATP decontamination, prime the injector with a 50% bleach (sodium hypochlorite) solution. Allow it to sit for one hour before rinsing. Flush the injector by ten times with sterile, distilled or deionized water. Proper luminometer care is important for low-assay background and precise ATP measurements.

2. Go to Select Protocol from the Home screen and follow the protocol wizard to select the preset Enliten ATP protocol. Enter the following: Luminescence; at the Preset tab, select Enliten ATP; Finish.

**Note:** If the instrument has been previously exposed to reagents containing ATP, such as the Promega LAR Reagent, the injector / tubing may be contaminated. It is preferable to use an injector/tubing set that has not been exposed to LAR when injecting rL/L reagent. For example, if injector #1 has been used for LAR injection, then use the #2 injector for ATP protocol.

3. The Instrument Control screen shows all the reading parameters: integration, each injector and injection volume, delay time, and that all the wells are selected. If desired, change the delay and integration time settings and save the changed protocol under a different name in the User protocol folder. The default parameters of the Enliten ATP protocol are 100  $\mu\text{L}$  rL/L Reagent injection; with two seconds delay and ten seconds integration time.
4. Select wells to be read on the "Plate Map" according to how the samples are loaded into the plate.
5. Prepare the injectors. Place the injector intake tubing into the bottle of rL/L Reagent.
6. Prime the injector using the Setup icon on the Read screen of Instrument control. Place the Waste Collection Tray in the instrument. Follow the step-by-step injector set-up wizard to prime.

**Note:** If it is important to conserve the reagent, a clean container (such as a cell/tissue culture plate) can be used for recovery.

7. Open the instrument door by using the Door icon on the touch screen. Place the plate with A1 well at the top right corner of the microplate sample tray. Close the door by using the Door icon.
8. Select the Start icon on the touch screen to start a read.
9. Refer to the on-screen Help topics, Quick Start Guide, or the Operating Manual for detailed instructions.

#### ATP Standard Curve

1. Manually add 10  $\mu\text{L}$  of assay buffer or ATP of a different concentration to a 96-well white microplate (E&K Scientific), according to the selected map in previous steps.
2. Run the Enliten ATP protocol.
3. Collect the RLU data from measuring ATP sample concentrations from  $1 \times 10^{-13}$ – $1 \times 10^{-7}$  M ATP.

#### Sample Analysis

1. Manually add 10  $\mu\text{L}$  of sample prepared in assay buffer to a 96-well white microplate (E&K Scientific), according to the previously selected map.
2. Run the Enliten ATP protocol.
3. RLU values measured by the GloMax<sup>®</sup> Multi Microplate Luminometer will appear on the Results screen of the touch-screen display immediately after each well is measured.
4. Once the measurements are complete, data can be transferred to an external computer for further data analysis in Excel by using the provided USB flash drive.
5. Remove the plate after completion.
6. Use the Reverse Purge function in the Setup wizard to recover unused reagent, if desired.
7. Use the Flush function in the Setup wizard to flush the injectors thoroughly after use.

8. Refer to the Operating Manual for detailed instructions on injector use.

#### RESULTS

**Sensitivity:**  $1 \times 10^{-18}$  moles (1 attomole) of ATP using the ENLITEN<sup>®</sup> ATP Assay

**Dynamic Range:** Up to six orders of magnitude dynamic range using the ENLITEN<sup>®</sup> ATP Assay

**Minimum Detection Limit:**  $2 \times 10^{-19}$  moles (0.2 attomole) of ATP using ENLITEN<sup>®</sup> ATP Assay.

#### CONCLUSION

The GloMax<sup>®</sup> Multi Microplate Luminometer offers superior sensitivity and dynamic range for luminescence-based ATP assay. The GloMax<sup>®</sup> Multi Microplate luminometer achieves its superior performance with a combination of unique detection and optical designs, premium components such as the photomultiplier tube (PMT), low-noise circuitry, and proprietary dual-masking system.

The modular approach of the GloMax<sup>®</sup> Multi Microplate Luminometer allows for instrument capability expansion as needs in the lab change. Fluorescence and/or absorbance detection modules as well as other accessories can be added after the initial purchase.

The superior performance, ease of use, and utmost flexibility of the GloMax<sup>®</sup> Multi Microplate make it an ideal microplate reader for today's life science laboratory.

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