

A GloMax[®] 96 Microplate Luminometer Method for the ENLITEN[®] ATP Assay

INTRODUCTION

The GloMax[®] 96 Microplate Luminometer combined with the ENLITEN[®] ATP Assay System Bioluminescence Detection Kit provides a sensitive, rapid method for measuring adenosine 5'-triphosphate (ATP). This reliable method for ATP detection is useful for studying enzymes that produce or degrade ATP. ATP detection also provides an indirect measurement of microbes, food residue, or other biological material.

The ATP-dependent oxidation of luciferin by luciferase produces light measured by the GloMax[®] 96 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.

As little as 1 attomole of ATP can be detected using the ENLITEN[®] ATP Assay and the GloMax[®] 96 Microplate Luminometer.

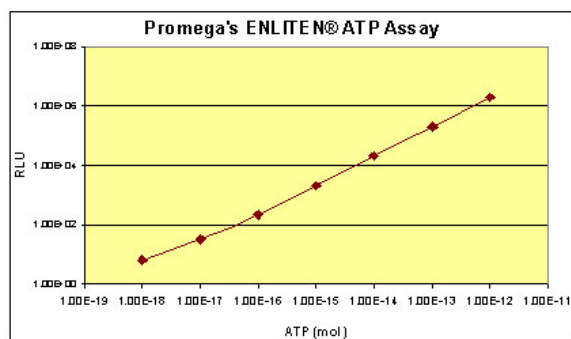


Figure 1. A standard curve is obtained using the GloMax[®] 96 Microplate Luminometer and the ENLITEN[®] ATP Assay System. An amount of 100 μ L rL/L Reagent was injected into sample wells of a 96 well plate containing 10 μ L of ATP Standard diluted in HEPES buffer.

When designing your ATP assay with the ENLITEN[®] kit, it is important to remember several key aspects of the luciferase reaction.

First, rL/L reacts optimally at pH 7.73 and 23 – 25°C. Salts and many nonionic chemicals will impair light production. Therefore, exercise caution when selecting buffers and ATP extractants for sample preparation. 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[®] 96 Microplate Luminometer
 - ❑ ENLITEN[®] 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 (1×10^{-7} M), 25 mL ATP-Free Water
 - ❑ 96-well, white microplates (E&K Scientific, 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°C prior to reconstitution. Store ATP Standards at -20°C .

EXPERIMENTAL PROTOCOL

1. 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.

- Equilibrate the sample buffer to room temperature.
- Lightly tap the vial of rL/L Reagent before opening to ensure the lyophilized material collects at the bottom of the vial.
- Transfer the vial contents of rL/L Reconstitution Buffer into the vial of the rL/L Reagent.
- Replace the stopper and slowly invert the vial several times to dissolve the contents. Do not shake the Reagent bottle.

- Allow the reconstituted rL/L Reagent to stand at room temperature for one hour before use.

Note: Reconstituted rL/L Reagent may be kept for eight hours at room temperature. Store at -20°C in single-use aliquots for long-term storage.

- Prepare a 10-fold serial dilution of the ATP Standard (1×10^{-7} M) in Assay Buffer. Dilute ATP to 1×10^{-13} M.

2. Instrument Set Up

- For ATP decontamination, prime the injector with a 50% bleach (sodium hypochlorite) solution. Allow it to sit for one hour. Flush the injector 10 times with sterile, distilled or deionized water. Proper luminometer care is important for low-assay background and precise ATP measurements.

Note: If the instrument has been previously exposed to reagents containing ATP, such as the Promega LAR Reagent, the injector or tubing may be contaminated. It is preferable to use an injector or 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 Assay protocol.

- Double click on the GloMax[®] 96 icon to start the software.
- Click on "Create New Protocol" from the Welcome to Veritas dialog box.
- Set up the protocol with the following parameters: one injector (#2); 100 μL injection volume; 2 seconds delay; and 10 seconds integration time. Also setup the plate map according to the samples loaded onto the plate. Once you have made your choices, click the Apply Changes button to accept changes or the Save Protocol As button to save the protocol. You can return to this screen

by clicking Options from the Main Dialog Box.

- Enter your information into the Experiment, Operator, Plate No., and Notes fields in the Main Dialog Box.
- Prepare the injectors. Place the injector intake tubing into the bottle of rL/L Reagent. Prime both injectors using the Prime tab located on the Main Dialog Box.

3. ATP Standard Curve

- Manually add 10 μL of Assay Buffer or ATP with a different concentration to a 96-well, white microplate (E&K Scientific, EK-25075), according to the selected map in previous steps.
- Place the plate with the A1 well at the top right corner of the microplate sample tray.
- Select the Start icon within the Main Dialog Box to start a read.
- Collect RLU data from measuring ATP sample concentrations from 1×10^{-13} to 1×10^{-7} M.

4. Sample Analysis

- Manually add 10 μL of sample prepared in Assay Buffer to a 96-well, white microplate (E&K Scientific, EK-25075), according to the previously selected map.

Note: For maximum reproducibility, equilibrate samples to room temperature before adding reagents.

- Insert the plate into the GloMax[®] 96 Microplate Luminometer and click Start to begin an assay. RLU values measured by the GloMax[®] 96 Microplate Luminometer will appear in an Excel spreadsheet after all the selected wells in each row have been read. Once the

measurements are complete, access Excel for data analysis.

- Remove the microplate after the measurements are complete.
- Choose the Reverse Purge tab to return unused rL/L Reagent to the bottle.
- Flush injectors thoroughly after each use.
- Refer to the Operating Manual for detailed instructions on injector use.

RESULTS

Sensitivity: 1×10^{-18} moles (1 attomole) of ATP using the Promega ENLITEN[®] ATP Assay

Dynamic Range: Up to 6 orders of magnitude dynamic range using the Promega ENLITEN[®] ATP Assay

Minimum Detection Limit: 5×10^{-19} moles (0.5 attomole) of ATP using the Promega ENLITEN[®] ATP Assay

CONCLUSION

The GloMax[®] 96 Microplate Luminometer offers superior sensitivity and dynamic range for luminescence-based ATP assay. The GloMax[®] 96 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 superior performance and ease of use of the GloMax[®] 96 Microplate Luminometer make it ideal for measurement of ATP levels using luminescence.

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