A 20/20^n Luminometer Method for

ENLITEN® ATP Assay System Bioluminescence Detection Kit for ATP

1. INTRODUCTION

The Turner BioSystems 20/20^n Luminometer combined with the Promega ENLITEN® ATP Assay System Bioluminescence Detection Kit provides a sensitive, rapid method for measuring adenosine 5'-triphosphate (ATP). A 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 20/20^n 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 20/20^n can detect attomole levels of ATP. The limit of detection for ATP with the ENLITEN® detection assay is 3 attomoles or 1.5 femtograms (Figure 1).

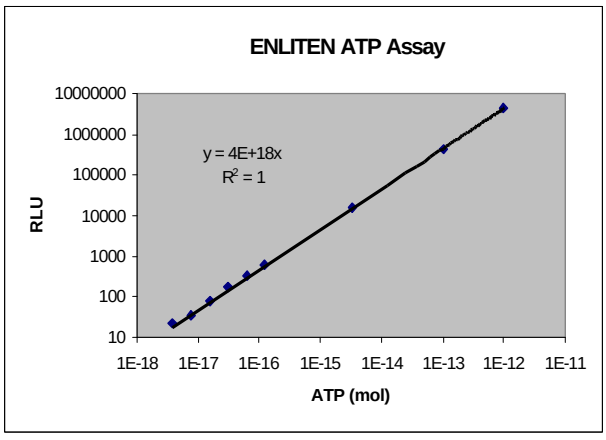

Figure 1. *A standard curve obtained using the 20/20^n Luminometer and the ENLITEN® ATP Assay System. 10 µL of ATP Standard diluted in HEPES buffer was added to a test tube containing 100 µL rL/L Reagent.*

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

First, the rL/L reacts optimally at a pH of 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 the sample preparations. It is also recommended to check for ATP contamination in your assay buffer by comparing the RLU values obtained with your assay buffer and rL/L to those of ATP-Free Water.

Within your sample, there may exist several different ATP stores. For example, in cell preparations, ATP may be present in the media. Treatment of cells may alter the amount of ATP in the media.

If you wish to measure the ATP content in microorganisms or cells, you will need to extract the ATP before analysis. Trichloroacetic acid (TCA) is recommended because it releases ATP from cells and inactivates ATP-degrading enzymes. Because TCA inhibits the luciferase reaction, it is important to determine the minimum amount of TCA necessary. Generally, 0.5% to 2.5% TCA (final concentration) is sufficient for ATP extraction from bacteria and eukaryotic cells.

Preparing a standard curve is a useful tool for proper ATP analysis of your samples. A standard curve should be prepared daily or whenever a new aliquot of the rL/L Reagent is used. The standard curve should include varying concentrations of ATP diluted in your assay buffer. The composition of this buffer should be identical to the composition of the buffer used in your assay to prepare your samples.

Keeping these considerations in mind will help you obtain the most accurate ATP analysis.

2. MATERIALS REQUIRED

From Turner BioSystems:

- 20/20^{n} Luminometer (P/N 2030-000)
- Microfuge Tube Holder

From Promega:

- ENLITEN® ATP Assay System Bioluminescence Detection Kit for ATP (P/N FF2000) containing 1 vial rLuciferase/Luciferin (rL/L) Reagent, 12 mL Reconstitution Buffer, 1 vial ATP Standard (1x10^{-7} M), 25 mL ATP-free Water

Other Materials:

- Adjustable p1000 Volume Pipetter and Tips
- Adjustable p200 Volume Pipetter and Tips
- Assay buffer
- 1.5 mL microfuge tubes
- Test tube rack
- Nitrile, vinyl, or latex gloves

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

Note: Individuals sensitive to latex should use vinyl or nitrile gloves.

3. REAGENT AND STANDARD PREPARATION

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

3.1 Equilibrate the sample buffer to room temperature.

3.2 Lightly tap the vial of the rL/L Reagent before opening to ensure the lyophilized material collects at the bottom of the vial.

3.3 Transfer the contents of the vial of rL/L Reconstitution Buffer to the vial of the rL/L Reagent.

3.4 Replace the stopper and slowly invert the vial several times to dissolve the contents. Do not shake the reagent bottle.

3.5 Allow the reconstituted rL/L Reagent to stand at room temperature for 1 hour before use.

Note: Reconstituted rL/L Reagent may be kept for 8 hours at room temperature. Store at –20°C in single-use aliquots for long-term storage.

3.6 Prepare a 10-fold serial dilution of the ATP standard (1x10^{-7} M) in assay buffer. Dilute to 1x10^{-11} M ATP.

4. INSTRUMENT SET-UP

4.1 Turn ON the 20/20^{n}. A warm-up period of 5 minutes is recommended but not necessary.

4.2 Touch the “Protocol” button on the bottom navigation bar.

4.3 Select the “ENLITEN” protocol. The next screen is the “Parameters” screen, which lists the optimal conditions for measuring ATP with ENLITEN. These options are preprogrammed. No adjustments are necessary. Touch “OK” to go to the “Home” screen.

5. ATP STANDARD CURVE

5.1 Add 10 µL of assay buffer to a microfuge tube containing 100 µL of rL/L Reagent.

5.2 Immediately place the microfuge tube into the 20/20^{n} Luminometer and close the lid.

5.3 Touch “Measure Luminescence” to begin measurement. The luminometer will measure the sample for 10-second integration period.

5.4 Record the value and repeat as necessary to obtain your background level on your assay buffer. Subtract the average background value from all subsequent measurements.

5.5 From the dilutions obtained in step 3.6, add 10 µL of the lowest concentration (1x10^{-11} M) of ATP to a microfuge tube containing 100 µL rL/L Reagent.

5.6 Immediately place the microfuge tube into the 20/20^{n} Luminometer and close the lid.

5.7 Touch “Measure Luminescence” to begin measurement.

5.8 Record this value and repeat with the next concentration of ATP. Continue for a total of six concentrations from $1x10^{-12}$ to $1x10^{-7}$ M ATP.

6. SAMPLE ANALYSIS

6.1 Add 10 µL of your sample prepared in the assay buffer to a microfuge tube containing 100 µL rL/L reagent.

6.2 Touch “Measure Luminescence”.

6.3 Record the value and repeat steps 6.1–6.2 for your remaining samples.

6.4 Plot the RLU values of your samples along with the RLU values obtained during the standard curve procedure to determine the concentration of ATP in your samples.

7. ABOUT PROMEGA CORPORATION

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