GenieGlow ATP Assay Kit (luminescence)(BN01006)

(Catalog BN01006 -100, -1000; Store kit at –20°C)

I. Introduction:

Adenosine-5'-triphosphate (ATP) is a central molecule in the chemistry of all living things and is used to monitor many biological processes. An accurate, reliable method to detect minute ATP levels such as the Luciferase/Luciferin system has broad application. Conventional Luciferase/Luciferin ATP detection systems are unstable since luciferase loses activity rapidly. At Assay Genie, we have developed a highly stable Luciferase; a genetically modified variant derived from the Luciferase of *Diaphanes pectinealis* (Chinese Firefly) endemic to Yunnan province, China. We designate our recombinant highly stable luciferase rLucHS. Compared to the normal phenotype of *Photinus pyralis*, rLucHS provides enhanced stability, excellent sensitivity, and a broader and more physiologically relevant effective pH range. At all pH's below ~ 8.2, rLucHS has a significantly higher relative activity than *Photinus* luciferase and is stable for weeks at room temperature and > 60 minutes at 37°C. Using the protocol outlined here, the quantitation range is between approximately 1 nmol to 10 fmol/assay. The specific activity of rLucHS is ~ 5 x 10^{11} RLU/mg protein. The assay can be fully automated for high throughput (1 sec/sample) and is extremely sensitive and is ideal for detecting ATP production or consumption in a variety of processes and enzymatic reactions.

Luciferin + ATP + O_2 $\xrightarrow[Luciferase]{Mg+2}$ Oxyluciferin + AMP + Pyrophosphate + CO_2 + Light

II. Kit Contents:

Component	BN01006 -100	BN01006 -1000	Cap Code
10X GenieGlow Reaction Buffer	20 ml	20 ml	WM
GenieGlow Reconstitution Buffer	11 ml	11 ml	NM
GenieGlow Enzyme Mix (lyophilized)	1 vial	1 vial	Green
ATP Standard (MW 551) (lyophilized)	1 vial	1 vial	Yellow

III. Reagent Reconstitution and General Considerations:

- Read the entire protocol before using this kit. Best results are achieved when all steps are performed in subdued lighting.
- Reconstitute the Enzyme Mix with 11 ml Reconstitution Buffer. Mix by gentle pipetting until completely dissolved. The reconstituted enzyme is stable for up to 2 months at 4°C, or longer at -20°C. Protect from light.
- Prepare ATP Standard by reconstituting the ATP vial with 100 µl dH_2O to generate a 10mM ATP stock solution. Aliquot and freeze; stable for several weeks at -20°C.
- Prepare enough 1X Reaction Buffer for the number of samples to be measured. Each well requires 10 µl 10X Reaction Buffer and 90 µl dH_2O. Additional buffer is needed for treatment of samples prior to measurement (read below).
- Because of the high sensitivity of the ATP assay, avoid contamination with ATP from exogenous biological sources, such as bacteria, fingerprints, glassware, etc.
- The Assay kit is not only stable, but also significantly more sensitive than other kits used for cell viability assays. The method can detect less than 10 cells, but as a general guide, we recommend using 10^3 - 10^4 cells per assay.
- The assay gives the best results using either a single tube or a white walled 96-well luminometer plate (100 µl/well reaction volume is recommended).

VI. ATP Assay Protocol:

1. **Reaction Mix:** Mix enough reagent for the number of samples and standards to be analyzed. For each assay, mix:

1X Reaction Buffer 80 µl

Enzyme Mix 10 µl

Mix and let it sit at room temperature for 1-2 hours to decrease background before use.

2. **Sample Preparation:** Quickly homogenize 1 x 10^3 - 10^4 cells or 10 mg tissue in 100 µl of 1X Reaction Buffer. Pellet at max speed for 30 sec to remove debris. Liquid samples can be directly used or diluted with the GenieGlow Reaction Buffer.
3. **Standard Curve:** To calculate absolute ATP content in samples, an ATP standard curve should be generated. Add 10 µl ATP stock solution to 990 µl of dH_2O to make 10^{-4} M ATP solution, into a tube labeled S1, then make 3 - 5 more 10 fold dilutions (i.e. 10 µl + 90 µl Reaction Buffer to generate S2, S3, S4, containing 10^{-5}M, 10^{-6}M, 10^{-7}M ATP, etc.).
4. **Measurement *:** Add 90 µl of the Reaction Mix into a series of wells in 96-well plate for the standard and number of samples to be analyzed. Then add 10 µl of standard or sample into the respective wells. Mix properly & read luminescence (L). (10 µl of 10^{-4} M ATP gives 1 nmol per well, 10 µl of 10^{-7} M ATP gives 1 pmol per well, etc.)

Note: For measuring low levels of ATP, first read background luminescence (BL) after adding 90 µl Reaction Mix into the wells and then add 10 µl sample or Standard. Mix properly and read total luminescence (L). Subtract BL from L to correct background luminescence.

5. **Calculations:** Plot the standard curve. Apply sample RLU values to the Standard curve to get Sa pmol of ATP amount in the sample wells.

ATP concentration in samples:

C = Sa/Sv (pmol/µl or nmol/ml, or µM)

Where: **Sa** is ATP amount (in pmol) from standard curve.
Sv is sample volume (in µl) added into the sample wells.
ATP molecular weight: 507.18 g/mol.

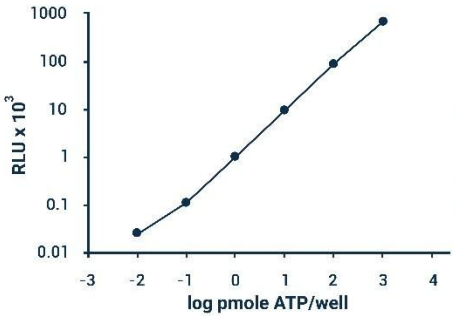

Fig. 1: Typical ATP Standard Curve (log/log)

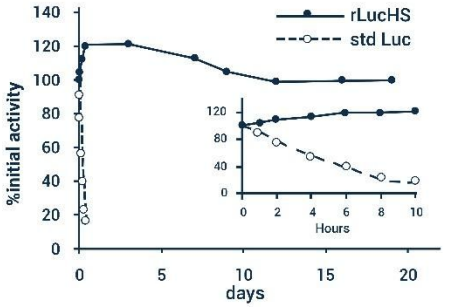

Fig. 2: rLucHS Stability at Room Temp.

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