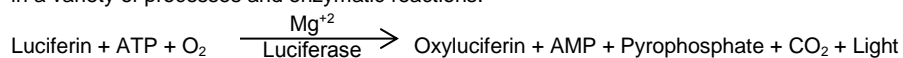


StayBrite™ Highly Stable ATP Bioluminescence Assay Kit

(Catalog #K791-100, -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 BioVision, 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 10¹¹ 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.



II. Kit Contents:

Component	K791-100	K791-1000	Cap Code	Part Number
	100 assays	1000 assays		
10X StayBrite™ Reaction Buffer	2.0 ml	20 ml	(-100) Red / (-1000) WM	K791-XX-1
StayBrite™ Reconstitution Buffer	1.1 ml	11 ml	(-100) Clear / (-1000) NM	K791-XX-2
StayBrite™ Enzyme Mix (lyophilized)	1 vial	1 vial	(-100) Green / (-1000) Amber	K791-XX-3
ATP Standard (MW 551) (lyophilized)	1 vial	1 vial	(-100) Yellow / (-1000) Yellow	K791-XX-4

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 (-100 assays) with 1.1 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.
- Reconstitute the Enzyme Mix (-1000 assays) 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 dH₂O to generate a 10 mM 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 dH₂O. 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 10³ - 10⁴ 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 (RT) for 1-2 hours to decrease background before use.

2. **Sample Preparation:** Quickly homogenize 1 x 10³ - 10⁴ 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 StayBrite™ Reaction Buffer.
3. **Standard Curve:** To calculate the absolute ATP content in samples, an ATP Standard Curve should be generated. Add 10 µl ATP stock solution to 990 µl of dH₂O 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:

$$C = \text{Sa/Sv} \quad (\text{pmol}/\mu\text{l or nmol}/\text{ml, or } \mu\text{M})$$

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

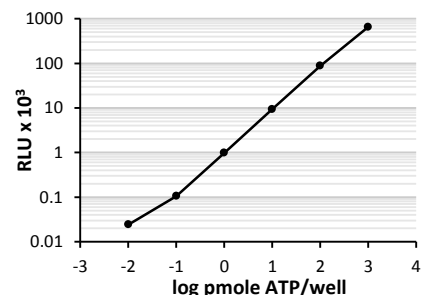


Fig. 1: Typical ATP Standard Curve (log/log).

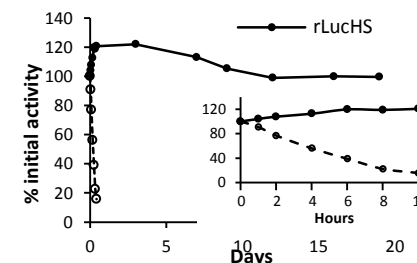


Fig. 2: rLucHS Stability at RT.

VI. Related Products:

- ATP and ADP Colorimetric/Fluorometric Assay Kit s (Cat. No. K354-100, K355-100)
- ADP/ATP Ratio Assay Kit (K255-200)
- Bioluminescent Cytotoxicity Assay Kit (K312-500)
- Luciferase Polyclonal Antibody (Cat. No. 3848-500)
- Luciferase Reporter Assay Kit (K801-200)
- StayBrite™ D-Luciferin, Sodium Salt (K7902-100, -1G, -10G)
- StayBrite™ Highly Stable Luciferase/Luciferin Reagent (Cat. No K790-100, -1000, -10000)
- StayBrite™ Highly Stable Recombinant Luciferase (Cat. No. 7901-150)

FOR RESEARCH USE ONLY! Not to be used on humans.