# Product Information

## StayBrite Highly Stable ATP Bioluminescence Assay Kit

### I. Kit Contents:

| Components | K2180-100<br>100 assays | Cap Color | Part Number |
|---|---|---|---|
| 10X StayBrite™ Reaction Buffer | 2.0 ml | Purple | K2180-C-1 |
| StayBrite™ Reconstitution Buffer | 1.1 ml | Clear | K2180-C-2 |
| StayBrite™ Enzyme Mix (lyophilized) | 1 vial | Green | K2180-C-3 |
| ATP Standard (MW 551) (lyophilized) | 1 vial | Yellow | K2180-C-4 |

### II. Introduction:

Luciferase is a useful tool for sensitive measurement of ATP levels, but the conventional method are usually unstable. The StayBrite Highly Stable ATP Bioluminescence Assay Kit utilize a highly stable Luciferase (rLucHS) which has enhanced stability and sensitive with relatively more effective pH range. rLuckHS is prominently more active than Photinus luciferase and is stable for weeks at room temperature and over 60 mins at 37ÁC. The detection limit can be as low as 10 fmol/assay (1 nM). rLucHS specific activity is ~ $5x10^{11}$RLU/mg protein. This assay can also be fully automated for high throughput (1 second/sample) and is highly sensitive ATP production or consumption in various enzymatic reactions.

### 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 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.

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 -20ÁC.

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 StayBrite™ 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 10-5M, 10-6M, 10-7M 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.

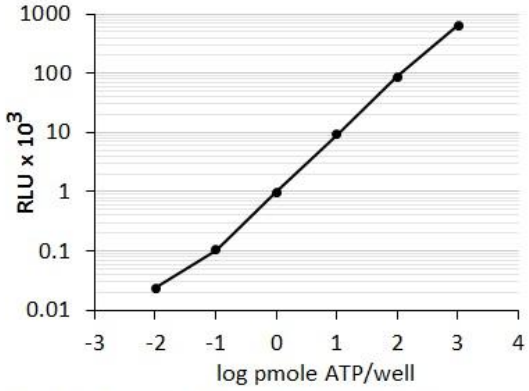


Fig. 1: Typical ATP Standard Curve (log/log).

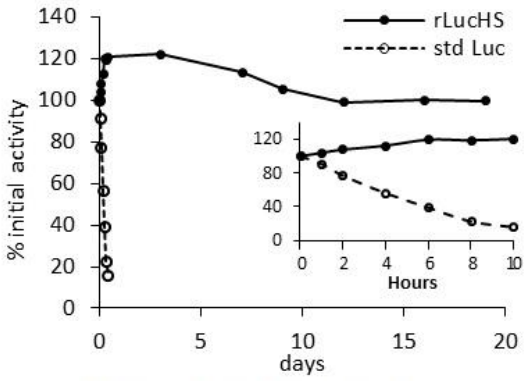


Fig. 2: rLucHS Stability at Room Temp.

**For research use only! Not to be used in humans.**

## Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com