ab287863 – Highly Stable ATP Assay Kit

A highly sensitive assay ideal for the luminescence detection of ATP production or consumption in a variety of processes and enzymatic reactions.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab287863

Storage and Stability

The entire kit may be stored at -20°C, protected from light.

Materials Supplied

Item	100 tests	1000 tests	Storage Condition
10X StayBite Reaction buffer	2 ml	20 ml	-20°C
ATP Standard (MW 551) (Lyophilized)	1 vial	1 vial	-20°C
StayBite Enzyme Mix (Lyophilized)	1 vial	1 vial	-20°C
StayBite reconstitution buffer	1.1 ml	11 ml	-20°C

Reagent Preparation

- 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.

Standard Preparation

- 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).

Δ Note: 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).

Assay Procedure

Reaction Mix:

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

Item	Reaction Mix
1X Reaction Buffer	80 µl
Enzyme Mix	10 µl

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

Sample Preparation:

1. Quickly homogenize 1 x 103- 104 cells or 10 mg tissue in 100 µl of 1X Reaction Buffer.
2. Pellet at max speed for 30 sec to remove debris. Liquid samples can be directly used or diluted with the StayBriteReaction Buffer.

Standard Curve:

1. To calculate the absolute ATP content in samples, an ATP Standard Curve should be generated.
2. 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 10-5M, 10-6M, 10-7M ATP, etc.).

Measurement

1. 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.
2. 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.)

Δ 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.

Calculation

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** = ATP amount (in pmol) from the Standard curve.
Sv = sample volume (in µl) added into the sample wells.

ATP molecular weight: 507.18 g/mol.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)