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CheKine™ Luminescent ATP Detection Assay

Cat #: KTB1019 Size: 48 T/48 S 96 T/96 S

	Luminescent ATP Detection Assay		
REF	**Cat #:** KTB1019	LOT	**Lot #:** Refer to product label
	Detection range: 1 nM-10 μM		**Sensitivity:** 1 nM
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacterium		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

In the presence of ATP, magnesium ions and oxygen, Luciferase (also known as Firefly Luciferase) can catalyze firefly luciferin to Oxyluciferin, which generate light signals during the oxidation of luciferin. In a certain range, the fluorescence intensity is proportional to the concentration of ATP.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Assay Buffer	7 mL	14 mL	4℃
Firefly Luciferase Substrate	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Luciferase	8.75 μL	17.5 μL	-20℃
ATP Standard (5 mM)	500 μL	500 μL	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Cell culture plate, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, 96-well black plate or 96-well white plate
- Luminometer or multimode reader

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Place on ice for use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Assay Buffer has certain irritation, so personal protection is recommended during use.

Reaction Buffer: Prepare before use, dissolve the Firefly Luciferase Substrate with the Assay Buffer, then transfer the dissolved Firefly Luciferase Substrate and Luciferase to the bottle containing Assay Buffer, mix well to obtain Reaction Buffer, and then pack according to usage requirements and stored at -20℃, protected from light. Equilibrate to room temperature before use.

Note: Reaction Buffer cannot be repeatedly freeze-thawed. If it is used less in a single experiment, it is recommended to pack it into small sizes according to the amount used in a single experiment. Store at -20℃, protected from light, recommended for use within 3 months, store at -80℃, protected from light, effective for 12 months.

ATP Standard (5 mM): Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

100 μM ATP Standard: Prepare before use, add 20 μL ATP Standard (5 mM) was to 980 μL Extraction Buffer, mix well, and placed on ice for use.

Standard curve setting: Dilute 100 μM standard with Extraction Buffer to an appropriate concentration gradient, depending on the concentration of ATP in the sample. The concentrations of 0, 0.001, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM can be set for the initial test, and the concentration range of the standard can be adjusted appropriately according to the concentration of ATP in the sample in subsequent experiments.

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

1. Serum or plasma Samples: According to the ratio of 0.1 mL serum or plasma add 0.9 mL Extraction Buffer, useing a pipette to repeatedly blow and mix well, Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant, placed on ice for detection.
2. Animal Tissue or Plant Tissue Samples: According to the ratio of 0.1 g tissue add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant, placed on ice for detection.
3. Adherent Cells: Discard the culture medium, According to the ratio of per well of the 6-well plate add 200 μL Extraction Buffer, use a pipette to repeatedly blow or shake the culture plate to allow the Extraction Buffer to fully contact and lyse cells. Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant, placed on ice for detection.
4. Suspension Cells: Collect 5×10^6 cells into centrifuge tubes, discard the supernatant after centrifugation. Add 1 mL Extraction Buffer, Using a pipette to repeatedly blow and lyse cells. Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant, placed on ice for detection.
5. Bacteria Samples: Collect 5×10^6 bacteria into centrifuge tubes, discard the supernatant after centrifugation. Add 1 mL Extraction Buffer. Ultrasonic wave breaks Bacteria in ice bath 1 min (power 20% or 200w, ultrasonic wave 2 s, interval 1 s, repeat 20 times). Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant, placed on ice for detection.

Note:

(1) ATP, especially ATP in the lysed sample, is not very stable at room temperature and needs to be operated at 4℃ or on ice. The extracted sample needs to be detected within 6 h.

(2) The cell or tissue samples obtained by lysis with the Extraction Buffer in this kit can not only be used for ATP detection, but also for protein concentration detection, SDS-PAGE or Western detection of some conventional more soluble proteins. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Add 100 μL Reaction Buffer into each detection well of 96-well black plate or 96-well white plate. Incubate at room temperature for 3-5 min to consume all background ATP, thereby reducing the background. 10-20 detection wells can be added to the 100 μL Reaction Buffer at one time to save time.
2. Add 20 μL sample or standard into each detection well, quickly mix, and immediately detect the chemiluminescence value in the luminometer or multimode reader.

Note:

(1) The number of one-time detection wells (including standard wells) must be controlled under 20.

(2) It is recommended to do a pre-experiment with 2-3 samples. According to the results of the pre-test, the concentration of the standard substance should be adjusted appropriately.

(3) The volume of the sample can be adjusted within the range of 10-100 μL. If the concentration of ATP in the sample is relatively low, a sample of 100 μL can be added, if the concentration of ATP in the sample is relatively high, a smaller volume of the sample can be added, and the same volume needs to be used for the standards. If the concentration of ATP in the sample is particularly high, the sample can be diluted with the Extraction Buffer before determination. When the 10-100 μL standards is added, the kit has a good linear relationship in the concentration range of roughly 1 nM-10 μM.

(4) For some special tissues or samples, if the detected ATP level is found to be significantly lower than expected, part of the sample can be boiled for 2 min after lysis of the sample and before centrifugation to fully release ATP. The protein in the sample is denatured after boiling and can be precipitated in subsequent centrifugation steps, so the boiled sample cannot be used for protein concentration determination, SDS-PAGE and Western assays. The remaining samples can be used for protein concentration determination, SDS-PAGE, and Western assays.

Precautions

1. Due to the influence of temperature on enzyme reactions, solution need to be equilibrated to room temperature before testing.
2. When mixing with a pipette, be careful to absorb and beat to avoid bubbles.
3. During testing, a 96-well black or white plate should be used to prevent interference from adjacent wells.
4. The maximum wavelength of bioluminescence catalyzed by firefly luciferase is 560 nm.

Data Analysis

The measured chemiluminescence RLU values of Standard Well and Test Well should minus the absorbance of Blank Well, that is, $\Delta RLU_{Standard}=RLU_{Standard}- RLU_{Blank}$, $\Delta RLU_{Test}=RLU_{Test}- RLU_{Blank}$.

1. Drawing of standard curve

With the concentration of the standard as the y-axis and the $\Delta RLU_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔRLU_{Test} into the equation to obtain the y value (nM)

2. Calculate the ATP content

(1) Calculated by volume of Liquid samples

ATP (nmol/mL) $=(y\div1,000\times V_{Sample})\div(V_{Extract}\div V_{Sample\ Total})\times n$**$=0.01\times y\times n$**

(2) Calculated by weight of samples

ATP (nmol/g) $=(y\div1,000\times V_{Sample})\div(W\times V_{样}\div V_{Sample\ Total})\times n$**$=0.01\times y\times n$**

(3) Calculated by protein concentration

ATP (nmol/mg prot)$=(y\div1,000\times V_{Sample})\div(V_{Sample}\times Cpr)\times n$**$=0.001\times y\div Cpr\times n$**

(4) Calculated by bacteria or cell number

ATP (nmol/10^4 Cells)$=(y\div1,000\times V_{Sample})\div(500\times V_{Sample}\div V_{Sample\ Total})\times n$**$=0.001\times y\div500\times n$**

Where: 1,000: 1 nM=10^{-3} nmol/mL; V_{Sample}: Sample volume added, 0.01 mL; $V_{Extract}$: Volumeof liquid sample added during extraction, 0.1 mL; $V_{Sample\ Total}$: Volume of samples, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, 0.1 g ; 500: Total number of bacteria or cells, 5×10^6; n: Dilution factor.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

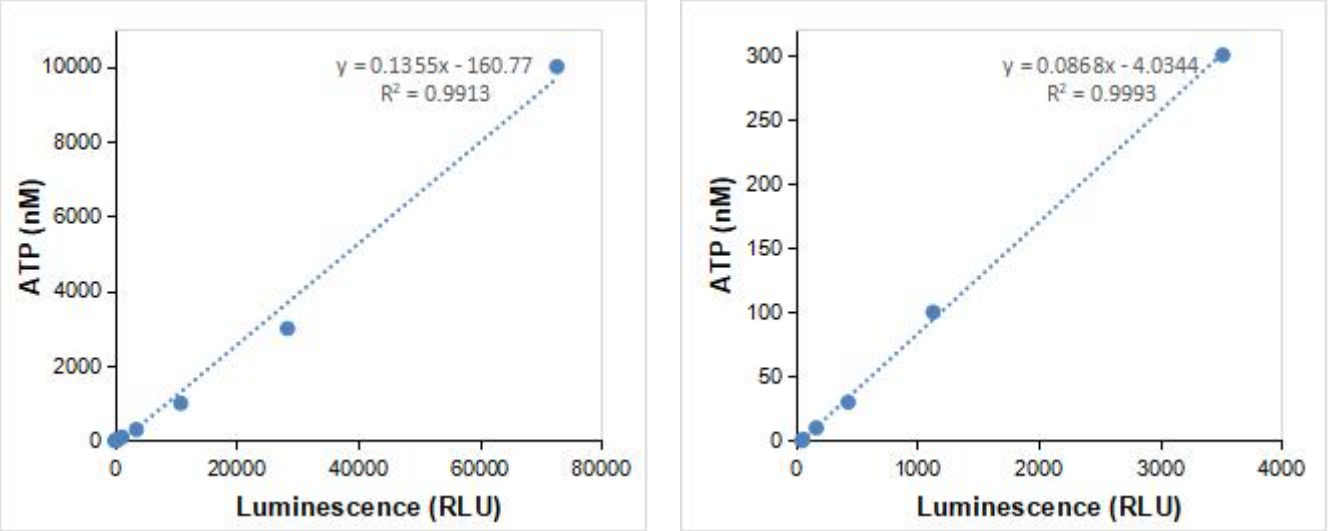

Figure1. Standard Curve of ATP in 96-well black plate.

Recommended Products

Catalog No.	Product Name
KTA8010	Dual Luciferase Reporter Gene Assay Kit
KTA8011	Luciferase Reporter Gene Assay Kit
BMC1041	Luminescent Mycoplasma Detection Kit
PRP3004	Luciferase firefly

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.