ATP Detection Kit

Catalogue No.	Product Name	Size
K027	ATP Detection Kit	200 tests

Introduction

ATP Detection Kit is designed to measure ATP(adenosine 5'-triphosphate) levels in general solution, cells, or tissues. Cell and tissue samples can be cleaved in one step to complete sample preparation. The detection sensitivity is up to 1nM, chemiluminescence can be stable for up to 30 minutes, and the obtained samples can be simultaneously detected by Western.

This kit is developed based on the energy required by ATP when firefly luciferase (luciferase) catalyzed luciferase to produce fluorescence.When both luciferase and luciferin are in excess, fluorescence production is proportional to ATP concentration in a certain range of concentrations.This allows highly sensitive detection of ATP concentration in solution.

This kit has high sensitivity, wide linear range, good detection effect in the range of 1nM to 10 μ M. It can detect ATP concentrations as low as 1nM at a sample volume of 100 μ L.The concentration of ATP in conventional cell or tissue lysates is 0.1-1μM, and the intracellular ATP level of some common cells is about 10nmol/mg protein. And the concentration range of this kit is very wide, the upper limit of detection can be up to 10μM, and can form a good standard curve in the range of 1nM-10μM.

Usually 10-20 samples can be determined in 30-60 minutes.

Kit Components

Item	Size
ATP detection reagent	2 mL(0.5ml/ tube, 4 tubes in total)
ATP detection reagent diluent	20 mL
ATP standard solution(0.5mM)	0.1mL
Lysis buffer	100mL
Product Description	1copy

Storage and Stability

The kit should be stored at -20°C for 6 months or -70°C for 12 months. ATP detection reagent should be stored away from light.

Precautions for Use

ATP detection reagent contains luciferase, which is gradually inactivated by repeated freezing and thawing. Although repeated freezing and thawing of ATP detection reagent for 5 times has no significant impact on its detection effect, in order to achieve better use effect, it is suggested that users should not use the number of freezing and thawing more than 3 times. After the ATP detection reagent is diluted into ATP detection working solution, it is best to use it all at one time. It is not suitable to use it after frozen storage.

ATP, especially in the sample after lysis, is not stable at room temperature and needs to be operated at 4℃ or on ice. ATP is stable on ice for up to six hours.

This kit uses luminometer, or chemiluminescence (the instrument used to detect luciferase reporter genes). If you don't have a Luminometer, you can use a liquid scintillation spectrometer. The determination effect of liquid scintillation spectrometer depends on its detection sensitivity and accuracy.

It is recommended to use a 96-well white board or blackboard that is opaque between holes when using a multifunctional microplate reader that can detect chemiluminescence.If common transparent 96-well plate is used, special attention should be paid to setting spacing holes between detection holes to reduce mutual interference between adjacent holes.For transparent 96-well plate, a luminous hole can raise the RLU value of the upper and lower or left and right adjacent holes about 10-20% of the hole, the RLU value of the adjacent hole of the upper and lower or left and right interval of a hole about 1%-4%; For the same sample, the chemiluminescence reading of the bottom opaque 96-well white board can reach about 5-10 times that of the transparent 96-well white board, and about 3 times that of the bottom transparent 96-well white board (the measured data will vary with the 96-well board, testing instrument and sample, etc.).

The lysis buffer provided by this kit effectively lyses and releases ATP in common cultured cells and tissues. For some specific tissues or samples, if ATP levels detected are significantly lower than expected, part of the sample can be boiled for 2 minutes after lysis and before centrifugation to fully release ATP. After boiling, the proteins in the sample will denaturate and precipitate in subsequent centrifugation steps, so the boiled sample cannot be used for protein concentration determination, SDS-PAGE and Western assays. Residual samples can be used for protein concentration determination, SDS-PAGE and Western detection.

This product is only limited to scientific research personnel, can not be used for clinical diagnosis or treatment, food or medicine, must not be stored in the ordinary residential.

For your safety and health, please wear clothes and wear disposable gloves.

Assay Procedure

1. Sample preparation

For adherent cells:

The culture medium was removed and the cells were lysed by adding lysate at the ratio of 200 microl lysate to each well of the 6-well plate (i.e. 1/10 of the amount of cell culture medium 2 mL). In order to lysis the cells adequately, a pipette can be used to repeatedly blow or shake the culture plate so that the lysate can fully contact and lysis the cells.Cells are usually cleaved immediately upon contact with lysate.Centrifugation at 12000g at 4℃ for 5 min after lysis, supernatant was taken for subsequent determination.

For suspended cells:

Centrifuge the cells with a centrifuge tube, discard the supernatant, and gently scatter the cells. Add the lysate at the ratio of 200 microliters of lysate to the number of cells in each well of the 6-well plate to lysate the cells.In order to fully dissociate the cells, the bottom of the centrifuge tube can be slammed or Vortex to fully contact and dissociate the cells.Cells are usually cleaved immediately upon contact with lysate.Centrifugation at 12000g at 4℃ for 5 min after lysis, supernatant was taken for subsequent determination.

For tissue samples:

The lysate is added at a rate of approximately 100-200 microliters per 20 mg of tissue and then homogenized using a glass homogenizer or other homogenizer.Adequate homogenization ensures complete cleavage of the tissue.Centrifugation at 12000g at 4℃ for 5 min after lysis, supernatant was taken for subsequent determination.

2. Standard dilution

The reagent to be used was dissolved in the ice bath and the ATP standard solution was diluted to an appropriate concentration gradient with lysis buffer. The specific concentration depends on the concentration of ATP in the sample. Initial detection can detect 0.01, 0.03, 0.1, 0.3, 1, 3 and 10μM, in subsequent experiments, the concentration range can be adjusted according to the concentration of ATP in the sample.

3. Reagent preparation

Prepare an appropriate amount of ATP detection working solution in proportion to the 100 μ l ATP detection working solution required for each sample or standard.

Melt the standby reagent in an ice bath.Take an appropriate amount of ATP detection reagent and dilute it with ATP detection reagent diluent in a ratio of 1:9. For example, 100 μ l ATP detection reagent is added into 900 μ l ATP detection reagent diluent to prepare 1 mL ATP detection working solution.The diluted ATP detection reagent is the working liquid for ATP detection in subsequent experiments. ATP detection solution can be temporarily stored in an ice bath.

4. Determination of ATP concentration

A. Add 100 μ l ATP detection working solution to test hole or test tube.Leave at room temperature for 3-5 minutes to allow all background ATP to be consumed and thus lower the background. 10-20 test holes or test tubes can be added with 100 μ l ATP detection working at one time to save time.

B. Add 20 μ l of sample or standard to the test hole or test tube and mix it quickly with a gun (micropipette). After an interval of at least 2 seconds, determine the RLU value or CPM with luminometer or liquid scintillation spectrometer.(Note: The sample volume can be adjusted within the range of 10-100 microliters. If the concentration of ATP in the sample is relatively low, 100 microliters can be added, if the concentration of ATP in the sample is relatively high, a smaller volume can be added, and the same volume should be used for the standard solution. If the concentration of ATP in the sample is particularly high, the sample can be diluted with lysis buffer and then determined. This kit has good linearity in the range of roughly 1nm-10 μM

when adding 10-100 microl of standard solution.

C. Calculate the concentration of ATP in the sample according to the standard curve.

D. In order to eliminate the error caused by the difference in protein content during sample preparation, the BCA protein assay kit an be used to determin the protein concentration in the samples. And then you convert your ATP concentration into nmol/mg protein.