Luminescent ATP Detection Assay Kit

Introduction

ATP is the most important source of energy in the cell and plays an important role in various intracellular processes. Abnormal changes in ATP levels are often associated with impaired cell functions, so it is important to detect ATP levels.

Luminescent ATP Detection Assay Kit is a kit that uses luciferase to detect ATP content in samples. The detection principle is that firefly luciferase can catalyze the substrate D-luciferin to emit light in the presence of ATP. When both firefly luciferase and D-luciferin are in excess, the luminescence signal produced is proportional to the concentration of ATP.

This kit can measure the amount of ATP within a solution, cell, or tissue. For cell or tissue samples, the kit provides a ready-to-use lysis buffer that can be used for ATP detection after simple lysis without the need for cumbersome steps such as trichloroacetic acid (TCA) or perchloric acid extraction or boiling after lysis. At the same time, the kit has high sensitivity and good linearity in the range of 1 nM-10 μM. The kit has been optimized to have a very stable luminescence signal for ATP detection, which can be stable for up to 30 minutes. In addition, the samples used in this kit can be used simultaneously for protein concentration determination, SDS-PAGE, and Western blot.

Components and Storage

Components	K2040-200 T
ATP Detection Reagent	4 x 500 μL
ATP Detection Diluent	20 mL
ATP Standard (0.5 mM)	100 μL
ATP Lysis Buffer	100 mL
Store the kit at -20°C, stable for 6 months. Or store the kit at -80°C, stable for 1 year. ATP Detection Reagent should be stored away from light.	

Protocol

1. **Sample Preparation:**

   1) **Adherent cells**: For 6-well plates, remove the culture medium, add 200 μL of ATP lysis buffer (1/10 of the volume of culture medium) per well and lyse cells on ice. Then 4°C, 12000 g centrifuge for 10 min to obtain the supernatant for detection.

2) **Suspension cells**: For 6-well plates, harvest the cells and centrifuge to remove the culture medium, add 200 μL of ATP lysis buffer (1/10 of the volume of culture medium) per well and lyse cells on ice. Then 4°C, 12000 g centrifuge for 10 min to obtain the supernatant for detection.

3) **Tissues:** 20 mg of tissue is taken and homogenized with 100-200 μL of ATP lysis buffer on ice. Then 4°C, 12000 g centrifuge for 10 min to obtain the supernatant for detection.

***Note:** Cell lysis or tissue homogenization needs to be performed on ice.

2. **Dilution of ATP Standard:** Thaw the following reagents on ice in advance. Gradient dilution of ATP Standard (0.5 mM) with ATP lysis buffer for subsequent preparation of the standard curve. The concentration of the ATP Standard can be set to 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM. The ATP standard concentrations can be adjusted depending on the special experiment.

3. **Preparation of the ATP detection working solution:** Thaw the following reagents on ice in advance, and then prepare the ATP detection working solution according to the table. 100 μL of ATP detection working solution is required for each sample or standard. The prepared ATP detection working solution can be stored temporarily on ice.

Sample Number	**10**	**20**	**50**
ATP Detection Reagent	100 μL	200 μL	500 μL
ATP Detection Diluent	900 μL	1.8 mL	4.5 mL

4. **Detection:**

1) Add 100 μL of ATP detection working solution to each test tube and leave it at room temperature for 3-5 minutes to consume all the ATP in the background. All the test tubes can be added at one time to save experimental time.

2) Then add 20 μL of sample or standard to each tube and mix quickly with a pipette. After an interval of at least 2 s, the detection is performed with a luminescence instrument or a multi-mode microplate reader with luminescence detection.

***Note:** Depending on the experiment, the sample and standard volume can be adjusted among 10-100 μL, but the sample and standard volume need to be kept consistent. If the sample concentration is particularly high, the sample can be diluted with ATP Lysis Buffer before detection.

3) Calculate the ATP concentration in the sample according to the standard curve.

4) In order to eliminate differences in the amount of protein in the preparation of the sample, the protein concentration in the sample can be measured with the BCA Protein Assay Kit and then converted to nmol/mg of protein.

***Note:** It is recommended to use the BCA Protein Assay Kit (Cat. No. K4101).

Note

1. ATP Detection Reagent contains firefly luciferase, so it is necessary to avoid repeated freeze-thaw cycles. The freeze-thaw cycles are recommended no more than 3 times. At the same time, try to prepare a fresh ATP detection working solution every time, do not cryopreservation.
2. ATP, especially in the lysed sample, is not stable at room temperature, so the experiment needs to be performed on ice or at 4°C. ATP can be stable on ice for up to 6 hours.
3. If using a multi-mode microplate reader, it is recommended to use a 96-well opaque plate, which can reduce interference between wells.
4. The ATP Lysis Buffer provided in this kit can effectively lyse common cells and tissues and release ATP. However, for some special samples, there may be cases where the ATP level is significantly lower than expected at the time of detection. This can be done after lysing the sample but before centrifugation, taking a portion of the sample and boiling it for 2 minutes to fully release the ATP. However, the protein in the sample is denatured and precipitated in subsequent centrifugation, so the boiled sample cannot be used for experiments such as protein concentration determination, SDS-PAGE, and Western blot. Partial samples that have not been boiled can be taken for these experiments.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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