

ATP Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: BC5475

Size: 100T/96S

Components:

Extract reagent: Liquid 110 mL×1. Storage at 2-8°C. Under low temperature conditions, there may be crystallization, put in a 60°C water bath to dissolve, does not affect the use of.

Reagent I: Liquid 20 mL×1. Storage at 2-8°C.

Reagent II: Powder×1. Storage at 2-8°C. Dissolved with 3.5 mL of distilled water before use. Dissolution of Reagent II could be promoted by heat, and the unspent reagent could be stored at 2-8°C for 4 weeks.

Reagent III: Liquid 4 mL×1. Storage at 2-8°C.

Reagent IV: Powder×2. Storage at -20°C. Each tube dissolved with 0.2 mL of distilled water before use. It can be divided into small tubules and preserved at -20°C for 2 weeks. Avoid repeating freeze/thaw cycles.

Reagent V: Powder×1. Storage at 2-8°C. Dissolved with 1 mL of distilled water before use, and the unspent reagent could be stored at -20°C for 4 weeks. Avoid repeating freeze/thaw cycles.

Reagent VI: Powder×2. Storage at -20°C. Dissolved with 0.25 mL of distilled water before use. It can be divided into small tubules and preserved at -20°C for 2 weeks. Avoid repeating freeze/thaw cycles.

Reagent VII: Liquid 4 mL×1. Storage at 2-8°C.

Standard: Powder×1 (5 mg ATP). Storage at -20°C. Dissolve in 0.826 mL of distilled water prepare as 10 μmol/mL standard solution before use. The unspent reagent could be stored at -20°C for 4 weeks.

Preparation of 0.4μmol/mL standard solution: Before use, 20μL 10 μmol/mL ATP standard solution and 480μL distilled water were mixed to prepare 0.4μmol/mL standard solution, which was used for the determination of standard tube.

Working solution: Reagent II, Reagent III, Reagent IV , Reagent V and Reagent VI = 0.2mL : 0.2mL : 0.02mL : 0.08mL : 0.02mL (0.52mL, about 10T). The reagent should be prepared just before use.

Product Description:

ATP (adenosine 5'-triphosphate) is found broadly in animals, plants, microorganisms and cultured cells, which is described as the energy currency in all living systems. Detecting the content of ATP and calculating the level of energy charge can reflect the state of energy metabolism.

Hexokinase (HK) catalyzes the synthesis of glucose and ATP into 6-phosphate glucose. 6-phosphate glucose dehydrogenase further catalyzes the dehydrogenation of glucose 6-phosphate and NADP to form NADPH. NADPH has a characteristic absorption peak at 340 nm, and the absorbance ratio of NADPH is

in direct proportion to contents of ATP.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath/constant temperature incubator, refrigerated centrifuge, transferpettor, micro quartz cuvette/96 well UV flat-bottom plate, ice, mortar/ homogenizer, distilled water and chloroform.

Procedure:

I. Sample preparation:

1. Serum (plasma):

According to the proportion, add the volume of serum (slurry) (mL): the volume of Extract solution (mL) is 1:5~10. It is suggested that add 1 mL of Extract solution to 0.1 mL of serum or plasma and shock blending. Centrifuge at 10000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant into another EP tube. Add 500 μL of chloroform into the supernatant and shock blending. Centrifuge at 10000 ×g for 3 minutes at 4°C to remove insoluble materials and take the supernatant ice for testing. (Note: Cannot be used for protein content determination).

2. Tissue:

According to the proportion, add the tissue weight (g): the volume of extract (mL) is 1:5~10. It is suggested that add 1 mL of extract solution to 0.1 g of tissue, and fully homogenized on ice. Centrifuge at 10000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant into another EP tube. Add 500 μL of chloroform into the supernatant and shock blending. Centrifuge at 10000 ×g for 3 minutes at 4°C to remove insoluble materials and take the supernatant ice for testing. (Note: Cannot be used for protein content determination).

3. Bacteria or cells:

Collect bacteria or cells into the centrifuge tube, centrifuge and discard supernatant. According to the proportion, add the bacteria or cells (10^4): the volume of Extract solution(mL) is 500~1000: 1. It is suggested that add 1 mL of Extract reagent to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cell (place on ice, ultrasonic power 200W, working time 2s, interval 1s, repeat for 20 times). Centrifuge at 10000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant into another EP tube. Add 500 μL of chloroform into the supernatant and shock blending. Centrifuge at 10000×g for 3 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing. (Note: Cannot be used for protein content determination).

II. Detect procedure:

1. Preheat spectrophotometer or microplate reader for more than 30 minutes, adjust the wavelength to 450 nm, set to zero with distilled water.

2. Preheat the reagent in a 37°C water bath/constant temperature incubator for more than 15min.

3. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Standard tube(S)	Blank tube (B)
Sample	20	-	-
Standard solution	-	20	-
Distilled water	-	-	20
Reagent I	130	130	130
Working solution	50	50	50
Mix well and incubate in 37°C water bath/incubator for 1h			
Reagent VII	30	30	30

Thoroughly mixed, 200μL reaction liquid was absorbed into A microglass colorimetric dish or 96-well plate to determine the light absorption value at 450nm, denoted as A_T , A_S , A_B , calculated $\Delta A_S = A_S - A_B$, $\Delta A_S = A_S - A_B$ (blank tube and standard tube only need to be done 1-2 times).

III. Calculation:

1. Serum (plasma):

$$\text{ATP } (\mu\text{mol/mL}) = C_S \times \Delta A_T \div \Delta A_S \times (V_e + V_s/p) \div V_s/p = 4.4 \times \Delta A_T \div \Delta A_S$$

2. Sample weight:

$$\text{ATP } (\mu\text{mol/g fresh weight}) = C_S \times \Delta A_T \div \Delta A_S \times V_e \div W = 0.4 \times \Delta A_T \div \Delta A_S \div W$$

3. Calculated by protein concentration:

$$\text{ATP } (\mu\text{mol/mg prot}) = C_S \times \Delta A_T \div \Delta A_S \times V_s \div (V_s \times C_{pr}) = 0.4 \times \Delta A_T \div \Delta A_S \div C_{pr}$$

4. Bacteria or cells:

$$\text{ATP } (\mu\text{mol}/10^4 \text{ cell}) = C_S \times \Delta A_T \div \Delta A_S \times V_e \div N = 0.4 \times \Delta A_T \div \Delta A_S \div N$$

C_S : Standard concentration, 0.4μmol/mL;

V_e : Extract volume, 1 mL;

V_s/p : Serum (plasma) volume, 0.1 mL;

W : Sample weight, g;

V_s : Sample volume added into the reaction system: 0.02mL;

C_{pr} : Sample protein concentration, mg/mL;

N : Total number of cells or bacteria, 10^4 .

Note:

1. It is normal for the supernatant to be turbid after adding the extract and centrifugation.
2. The extraction process must be strictly carried out under ice bath conditions.
3. If $A > 1.5$. It is recommended to dilute the sample with distilled water for determination. Note that the formula is multiplied by dilution; If the absorption value is too low or close to blank, it is recommended to place it in a 37°C water bath/constant temperature incubator for 2h or longer before testing again, or

increase the sample size before testing, and pay attention to changing the calculation formula simultaneously.

4. The Extract reagent may crystallize, which can be dissolved in 60°C water bath without affecting the use at a low temperature.

Experimental example:

1. Take 0.108g of rabbit muscle, add 1 mL of Extract solution to homogenize in ice bath, centrifuge at 4°C and 10000g for 10 minutes, take the supernatant into another EP tube, add 500 μL of chloroform, mix well. After centrifuging at 4°C and 10000 g for 3 minutes, the supernatant is put on ice and operated according to the determination steps. The results showed that $\Delta A_T = 0.222 - 0.018 = 0.104$, $\Delta A_S = 0.466 - 0.118 = 0.348$. The content of:

$$\text{ATP } (\mu\text{mol/g weight}) = 0.4 \times \Delta A_T \div \Delta A_S \div W = 1.107 \mu\text{mol/g weight.}$$

2. Take 0.1 g of Allium sativum, add 1 mL of Extract solution to homogenize in ice bath, centrifuge at 4°C and 10000g for 10 minutes, take the supernatant into another EP tube, add 500 μL of chloroform, mix well. After centrifuging at 4°C and 10000g for 3 minutes, the supernatant is put on ice and operated according to the determination steps. The results showed that $\Delta A_T = 0.248 - 0.118 = 0.166$, $\Delta A_S = 0.466 - 0.118 = 0.348$. The content of:

$$\text{ATP } (\mu\text{mol/g weight}) = 0.4 \times \Delta A_T \div \Delta A_S \div W = 1.908 \mu\text{mol/g weight.}$$

References:

[1] Lin X F, Wu Y P, Cheng X J, et al. Measurement of adenosine phosphate in Phenol-TEA Pretreatment tobacco by UPLC[J]. Acta Tabacaria Sinica, 2014, 20(1): 26-31.

[2] Beutler E, Mathai C K. A comparison of normal red cell ATP levels as measured by the firefly system and the hexokinase system[J]. Blood, 1967, 30(3): 311-320.

Related Protects:

BC0060/BC0065 Na⁺K⁺-ATP Activity Assay Kit

BC0960/BC0965 Ca⁺⁺Mg⁺⁺-ATP Activity Assay Kit

Technical Specification:

The detection limit: 0.0026 μmol/mL

The linear range: 0.01953-3 μmol/mL