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: BC0305

Size: 100T/96S

Components:

Extract reagent: Liquid 110 mL×1. Storage at 4℃.

Reagent I: Liquid 20 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. 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 4℃ for 4 weeks.

Reagent III: Liquid 4 mL×1. Storage at 4℃.

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

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

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

Standard: Powder×1 (5 mg ATP). Storage at -20℃. 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℃ for 4 weeks.

Working solution: Reagent II, Reagent III, Reagent IV , Reagent V and Reagent VI are mixed by the volume ratio of 1 : 1 : 0.1 : 0.4 : 0.1(2.6mL, about 50T). 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, 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℃ 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℃ 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 8000 ×g for 10 minutes at 4℃ 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℃ 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℃ 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℃ 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 340 nm, set to zero with distilled water.

2. Dilute the 10 μmol/mL standard solution16 times to 0.625 μmol/mL standard with distilled water.

3. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Standard tube(S)
Sample	20	-
Standard solution	-	20
Reagent I	128	128
Working solution	52	52

Mix thoroughly and timing, detect the absorbance at 340 nm at the time of 10 seconds record as A1(10s). Then place the cuvette with the reaction solution in a 37℃ (mammals) or 25℃ (other species) water bath or incubator for 3 minutes. (If the microplate reader has the function of temperature control, the temperature can be adjusted to 37 or 25℃). Take it out and wipe it clean, then immediately measure the

absorbance at 3min 10s of final reaction which record as A2. ΔA(T)=A2(T)-A1(T), ΔA(S)=A2(S)-A1(S).

III. Calculation:

1. Serum (plasma):

   ATP (μmol/mL)=ΔA(T)÷(ΔA(S)÷C_S)×(Ve+Vs/p)÷Vs/p=6.875×ΔA(T)÷ΔA(S)

2. Sample weight:

   ATP (μmol/g fresh weight)=ΔA(T)÷(ΔA(S)÷C_S)×Ve÷W=0.625×ΔA(T)÷ΔA(S)÷W

3. Bacteria or cells:

   ATP (μmol/10^6 cell)=ΔA(T)÷(ΔA(S)÷C_S)×Ve÷5=0.125×ΔA(T)÷ΔA(S)

Cs: Standard concentration, 0.625 μmol/mL;
Ve: Extract volume, 1 mL;
Vs/p: Serum (plasma) volume, 0.1 mL;
W: Sample weight, g;
5: The total number of cells or bacteria, 5×10^6.

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, the sample can be determined after being appropriately diluted.
4. The Extract reagent may crystallize, which can be dissolved in 60℃ water bath without affecting the use at a low temperature.

Experimental example:

1. Take 0.1g of rabbit lung, add 1 mL of Extract solution to homogenize in ice bath, centrifuge at 4℃ and 8000g for 10 min, take the supernatant into another EP tube, add 500 μL of chloroform, mix well. After centrifuging at 4℃ and 10000 g for 3 min, the supernatant is put on ice and operated according to the determination steps. The results showed that $\Delta A_T=A_{T2}-A_{T1}$=0.0873-0.065=0.0223, $\Delta A_S=A_{S2}-A_{S1}$= =0.4368-0.1435=0.2933.

   The content of ATP (μmol/g mass) = $0.625\times\Delta A_T \div \Delta A_S \div W$ =0.625×0.0223÷0.2933÷0.1=0.475 μmol/g mass.

2. Take 0.1 g of Echinochloa crusgalli, add 1 mL of Extract solution to homogenize in ice bath, centrifuge at 4℃ and 8000g for 10 min, take the supernatant into another EP tube, add 500 μL of chloroform, mix well. After centrifuging at 4℃ and 10000g for 3 min, the supernatant is put on ice and operated according to the determination steps. The results showed that $\Delta A_T= A_{T2}-A_{T1}$=0.5351-0.4969=0.0382, $\Delta A_S= A_{S2}-A_{S1}$=0.4368-0.1435=0.2933.

   The content of ATP (μmol/g mass) = $0.625\times\Delta A_T \div A_S \div W$ =0.625×0.0382÷0.2933÷0.1=0.814 μmol/g mass.

3. Take 0.1 mL of rabbit serum, add 1mL of extract, shake fully, centrifuge at 4℃ and 10000g for 10 min; take the supernatant into another EP tube, add 500 μL of chloroform, shake fully, mix well. After centrifuging at 4℃ and 10000g for 3 min, the supernatant is put on ice for detection. $\Delta A_T = A_{T2}-A_{T1}$ ==0.0569-0.0449=0.012, $\Delta A_S = A_{S2}-A_{S1}$=0.4368-0.1435=0.2933

The content of ATP (μmol/mL) = $6.875\times\Delta A_T \div A_S$ =6.875×0.012÷0.2933=0.281 μmol/mL.

Recent Product Citations：

[1] Meixi Peng, Dan Yang, Yixuan Hou, et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKP and CS enhances hypoxic breast cancer cell invasion and metastasis. Cell Death and Disease. March 2019; (IF5.959)

[2] Yang Wang, Jianhang Jiao, Shanyong Zhang, et al. RIP3 inhibition protects locomotion function through ameliorating mitochondrial antioxidative capacity after spinal cord injury. Biomedicine & Pharmacotherapy. August 2019;116. (IF3.743)

[3] Luo M, Luo Y, Mao N, et al. Cancer-Associated Fibroblasts Accelerate Malignant Progression of Non-Small Cell Lung Cancer via Connexin 43-Formed Unidirectional Gap Junctional Intercellular Communication. Cellular Physiology and Biochemistry. November 2018.

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