ATP Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC5470

Size: 50T/48S

Components:

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

Reagent I: Liquid 45 mL×1. Storage at 2-8℃.

Reagent II: Powder×1. Storage at 2-8℃. Dissolved with 7mL of distilled water before use. Dissolution of Reagent II could be promoted by heat, and the unspent reagent could be stored at 2-8℃ for 4 weeks.

Reagent III: Liquid 8 mL×1. Storage at 2-8℃.

Reagent IV: Powder×3. 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 2-8℃. Dissolved with 3.2 mL of distilled water before use, and the unspent reagent could be stored at -20℃ for 4 weeks. Avoid repeating freeze/thaw cycles.

Reagent VI: Powder×3. 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.

Reagent VII: Liquid 12 mL×1. Storage at 2-8℃.

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.

Preparation of 0.3125μmol/mL standard solution: Before use, 20μL 10 μmol/mL ATP standard solution and 620μL distilled water were mixed to prepare 0.3125μmol/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 = 1mL :1mL : 0.1mL : 0.4mL : 0.1mL (2.6mL, 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, the

absorbance ratio of NADPH is in

direct proportion to contents of ATP.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath/constant temperature incubator, refrigerated centrifuge, transferpettor, 1 mL quartz cuvette, 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 solution(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℃ 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 before testing. (Note: Cannot be used for protein content determination).

3. Bacteria or cells:

Collecting bacteria or cells into the centrifuge tube, centrifugation 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 splitting bacteria and cell (placed on ice, ultrasonic power 20% or 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. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust the wavelength to 450 nm, set to zero with distilled water.

2. The reagent is placed in a 37℃ water bath/constant temperature incubator and preheated for more than 15minutes.

3. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Standard tube(S)	Blank tube (B)
Sample	100	-	-
Standard solution	-	100	-
Distilled water	-	-	100
Reagent I	650	650	650
Working solution	250	250	250
Mix well and incubate in 37°C water bath/incubator for 1h			
Reagent VII	150	150	150

Thoroughly mixed and measured the absorption value at 450nm in 1mL glass cupola, denoted as A_T, A_S, A_B, calculated $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$ (blank tube and standard tube only need to be done 1-2 times).

III. Calculation:

1. Serum (plasma):

   ATP (μmol/mL)= $C_S \times \Delta A_T \div \Delta A_S \times (Ve+Vs/p) \div Vs/p = 3.4375 \times \Delta A_T \div \Delta A_S$

2. Sample weight:

   ATP (μmol/g fresh weight)= $C_S \times \Delta A_T \div \Delta A_S \times Ve \div W = 0.3125 \times \Delta A_T \div \Delta A_S \div W$

3. Calculated by protein concentration:

   ATP (μmol/mg prot)= $C_S \times \Delta A_T \div \Delta A_S \times Vs \div (Vs \times Cpr) = 0.3125 \times \Delta A_T \div \Delta A_S \div Cpr$

4. Bacteria or cultured cells:

   ATP (μmol/10^4 cell)= $C_S \times \Delta A_T \div \Delta A_S \times Ve \div N = 0.3125 \times \Delta A_T \div \Delta A_S \div N$

   Cs: Standard concentration, 0.3125μmol/mL;
   Ve: Extract volume, 1 mL;
   Vs/p: Serum (plasma) volume, 0.1 mL;
   W: Sample weight, g;
   Vs: Sample volume added into the reaction system: 0.1mL;
   Cpr: 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 solution and centrifugation.
2. The extraction process must be strictly carried out under ice bath conditions.

3. If ΔA >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℃ 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℃ 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℃ and 10000g for 10 minutes, take the supernatant into another EP tube, add 500 μL of chloroform, mix well. After centrifuging at 4℃ and 10000 g for 3 minutes, the supernatant is put on ice and operated according to the determination steps. The results showed that ΔA_T=0.283-0.154=0.129, ΔA_S=0.569-0.154=0.415. The content of:

ATP (μmol/g weight) = 0.3125×ΔA_T÷ΔA_S÷W =0.899 μmol/g weight.

2. Take 0.1 g of *Epipremnum aureum*, add 1 mL of Extract solution to homogenize in ice bath, centrifuge at 4℃ and 10000g for 10 minutes, take the supernatant into another EP tube, add 500 μL of chloroform, mix well. After centrifuging at 4℃ and 10000g for 3 minutes, the supernatant is put on ice and operated according to the determination steps. The results showed that ΔA_T=0.387-0.154=0.233, ΔA_S= 0.569-0.154=0.415. The content of:

ATP (μmol/g weight) = 0.3125×ΔA_T÷ΔA_S÷W =1.58 μmol/g weight.

Technical Specification:

The detection limit: 0.0023 μmol/mL

The linear range: 0.0390625-2.5 μmol/mL

References:

[1] Lin XF, Wu YP, Chen XJ, et al. Determination of adenosine phosphate in tobacco leaf by UPLC with phenol-TEA pretreatment [J]. Acta tabacaria sinca, 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 th-e hexokinase system[J]. Blood, 1967, 30(3): 311-320.

Related Protects:

BC0060/BC0065 Na^+k^+-ATPase Assay Kit

BC0960/BC0965 $Ca^{++}Mg^{++}$-ATPase Assay Kit