ATP Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0300

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 50 mL×1	2-8℃
Reagent II	Powder×1	2-8℃
Reagent III	Liquid 8 mL×1	2-8℃
Reagent IV	Powder×3	-20℃
Reagent V	Powder×1	2-8℃
Reagent VI	Powder×3	-20℃
Standard	Powder×1	-20℃

Solution Preparation:

1. Extract solution: At low temperature, the extract may crystallize out, and it can be heated and dissolved in a water bath at 60℃, which does not affect the use.

2. Reagent II: Dissolved with 7 mL of distilled water before use. Heating can be performed in the preparing process of Reagent II so as to accelerate the dissolution. It can be stored for 4weeks at 2-8℃.

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

4. Reagent V: Dissolved with 3.2 mL of distilled water before use. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeating freeze/thaw cycles.

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

6. Standard: 5 mg ATP. Storage at -20℃. Dissolve in 0.826 mL of distilled water to prepare as 10 μmol/mL standard solution before use. It can be stored at -20℃ for 4 weeks.

7. Working solution: Reagent II: Reagent III: Reagent IV: Reagent V: Reagent VI =1: 1: 0.1: 0.4: 0.1 (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 (>98%, AR).

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 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 340 nm, set to zero with distilled water.

2. Dilution of standard solution: Take 100μL 10μmol/mL ATP standard solution, add 1.5mL distilled water, mix well, prepare 0.625μmol/mL standard solution for use. (In the experiment,

100μL is required for
each tube, so the large volume is prepared to reduce the experimental error.)

3. Reagent I 37℃ (mammals) or 25℃ (other species) preheat for 10min.

4. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Standard tube(S)
Sample	100	-
0.625 μmol/mL Standard solution	-	100
Reagent I	640	640
Working solution	260	260
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℃ (mammal) or 25℃ (other species) water bath or incubator for 3 minutes. Take it out and wipe it clean, then immediately measure the absorbance at 3minutes 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 cultured 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 solution and centrifugation.

2. The extraction process must be strictly carried out under ice bath conditions.

3. If ΔA(T)>1.1, it is recommended to dilute the sample with distilled water for determination. Note that the formula is multiplied by dilution times; If the absorption value is too low or close to blank, it is recommended to increase the sample size to determine, pay attention to the simultaneous modification of the calculation formula.

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

Technical Specification:

The detection limit: 0.0023 μmol/mL

The linear range: 0.0390625-2.5 μmol/Ml

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 centrifugation 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 $\Delta AT = A_{T2}-A_{T1} = 0.083-0.066 = 0.017$, $\Delta A_S = A_{S2}-A_{S1} = 0.430-0.191 = 0.239$.

The content of ATP (μmol/g mass) = $0.625\times\Delta A_T \div \Delta A_S \div W = 0.625 \times 0.017 \div 0.239 \div 0.1 = 0.445$ 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 centrifugation at 4℃ and 10000g for 3 min, the supernatant is put on ice and operated according to the determination steps. The results showed that $\Delta AT = A_{T2}-A_{T1} = 0.511-0.479=0.032$, $\Delta A_S = A_{S2}-A_{S1} = 0.430-0.191 = 0.239$,

The content of ATP (μmol/g mass) = $0.625\times\Delta A_T \div A_S \div W = 0.625 \times 0.032 \div 0.239 \div 0.1 = 0.837$ 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, 10000g After centrifugation at 4℃ and 10000g for 3 min, the supernatant was put on ice for detection. $\Delta AT = A_{T2}-A_{T1} = 0.051-0.038 = 0.013$, $\Delta A_S = A_{S2}-A_{S1} = 0.430-0.191 = 0.239$

The content of ATP (μmol/mL) = $6.875\times\Delta A_T \div A_S = 6.875\times0.013 \div 0.239 = 0.374$ μmol/mL.

Recent Product Citations:

[1] Li C, Zhang X, Yang B, Wei F, Ren Y, Mu W, Han X. Reversible Deformation of Artificial Cell Colonies Triggered by Actin Polymerization for Muscle Behavior Mimicry. Adv Mater. 2022 Aug;34(34):e2204039. doi: 10.1002/adma.202204039. Epub 2022 Jul 17. PMID: 35765153.

[2] Yan B, Liu C, Li H, Wen N, Jiao W, Wang S, Zhang Y, Zhang T, Zhang H, Lv Y, Fan H, Liu X. Reversal of HMGA1-Mediated Immunosuppression Synergizes with Immunogenic Magnetothermodynamic for Improved Hepatocellular Carcinoma Therapy. ACS Nano. 2023 May 23;17(10):9209-9223. doi: 10.1021/acsnano.3c00004. Epub 2023 May 10. PMID: 37162457.

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

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