# Electron transport chain Complex V Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/Microplate Reader

**Cat No:** BC1445

**Size:**100T/48S

**Components:**

**Extract solution:** Liquid 60 mL×1. Storage at 2-8℃.

**Reagent I:** Liquid 50 mL×1. Storage at 2-8℃.

**Reagent II:** Powder×1. Storage at -20℃. Dissolve with 1.11 mL of distilled water before use. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.

**Reagent III**: Liquid 6 mL×1. Storage at 2-8℃.

**Reagent IV:** Liquid 3 mL×1. Storage at 2-8℃.

**Reagent V:** Powder×1. Storage at 2-8℃. Dissolve with 10 mL of distilled water before use. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.

**Reagent VI:** Powder×1. Storage at 2-8℃. Dissolve with 10 mL of distilled water before use. The unused reagent can be stored at 2-8℃ for 4 weeks. Avoid repeated freezing and thawing.

**Reagent VII:** Liquid 10 mL×1. Storage at room temperature.

**Standard solution:** 1 mL×1, 10 μmol/mL phosphorus standard solution. Storage at 2-8℃. Dilute 40 times with distilled water to prepare 0.25 μmol/mL phosphorus standard solution before use.

**Phosphorus fixing reagent:** Prepare the reagent for determining phosphorus content: make solution as the volume ratio of **$H_2O$:** Reagent V: Reagent VI: Reagent VII =20mL:10mL:10mL:10mL(about 100 tubes). The prepared reagent shall be light yellow, if colorless means the reagent is fail, if blue means phosphorus pollution. Prepare the reagent when it will be use.

**Note:** It is better to use new beakers, glass rods and glass pipettes, or disposable plastic containers to avoid phosphorus pollution.

**Product Description:**

Mitochondrial Respiratory Chain Complex V also known as $F_1F_0$-ATP synthase, widely exists in mitochondria of animals, plants, microorganisms and cultured cells, and consists of two subunits $F_1$ and $F_0$.

The enzyme catalyzes the synthesis of ATP using the proton electrochemical gradient generated by respiratory chain, and also hydrolyzes ATP in a reversible process. In addition, complex V is also present in chloroplasts, heterotrophs and photosynthetic bacteria. Complex V is the key enzyme for ATP synthesis from mitochondrial oxidative phosphorylation and chloroplast photophosphorylation.

Complex V hydrolyzes ATP to produce ADP and Pi. Molybdenum blue can react with inorganic phosphorus, the reaction product can be detected by colorimetric assay at 660 nm and determine the Complex V activity indirectly.

**Reagents and Equipment Required but Not Provided:**

Spectrophotometer/Microplate Reader, Water Bath/Constant Temperature Incubator, Desk Centrifuge, Adjustable Transferpettor, Micro Glass Cuvette/ 96 Well Flat-Bottom Plate, Mortar/Homogenizer/Cell Ultrasonic Crusher, Ice and Distilled Water.

**Procedure:**

**I. Complex V extraction:**

Collecting 0.1 g of tissue or 5 million cells, add 1 mL of extract solution, grinding on ice with mortar/homogenizer. After centrifuge at 600 ×g for 10 minutes at 4℃, take the supernatant to other tube and centrifuge at 11000 ×g for 15 minutes at 4℃ to separate supernatant and sediment again. The supernatant can used to detect Complex V that leaking from mitochondria, which shows the effect of mitochondrial extraction. Add 600 μL of Extraction solution to the sediment, splitting with ultrasonication (power 200W, work time 5s, interval 10s, repeat 12 times), used to detect the enzyme activity of Complex V and protein content.

**II. Determination procedure:**

1) Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 660 nm, set zero with distilled water.
2) Enzymatic reaction.

| Reagent name (μL) | Contrast tube (Ac) | Test tube ($A_T$) | Standard tube ($A_S$) | Blank tube($A_b$) |
|---|---|---|---|---|
| Reagent II | 10 | 10 | - | - |
| Reagent III | 40 | 40 | - | - |
| Sample | - | 50 | - | - |
| Mix thoroughly, then place the reaction solution in a 37℃ (mammal) or 25℃ (other species) water bath for 30 minutes. | | | | |
| Reagent IV | 20 | 20 | - | - |
| Sample | 50 | - | - | - |
| Mix thoroughly, centrifuge at 8000 rpm for 10 minutes at room temperature, get supernatant. | | | | |

3) Phosphorus content detection.

| Reagent name (μL) | Contrast tube (Ac) | Test tube ($A_T$) | Standard tube ($A_S$) | Blank tube(Ab) |
|---|---|---|---|---|
| Supernatant | 40 | 40 | - | - |
| Standard solution(0.25 μmol/mL) | - | - | 40 | - |
| Distilled water | - | - | - | 40 |
| Phosphorus fixing reagent | 200 | 200 | 200 | 200 |

Mix thoroughly, then place the mix solution in a 40℃water bath for 10 minutes and soon take

200 μL

to detect the absorbance at 660 nm. $\Delta A=A_T$ - Ac; $\Delta As$=As - Ab. The standard curve and blank tube only need to be measured 1-2 times.

**Calculation:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme generates 1nmol of inorganic phosphorus per minute every mg tissue protein.

Complex V Activity (U/mg prot) = $\Delta A \div \Delta A_S \times C \times Vrv \times 1000 \div (Cpr \times Vs) \div T = 20 \times \Delta A \div \Delta A_S \div Cpr$

C: Standard concentration, 0.25 μmol/mL; 1000: 1 μmol = 1000 nmol; Cpr: Sample protein concentration (mg/mL), need to measure by yourself; Vs: Sample volume (mL), 0.05 mL; Vrv: Enzymatic reaction volume,0.12 mL; T: Reaction time (min), 30 minutes.

**Note:**

1. Take two or three different samples for prediction before test. Dilute supernatant with distilled water if the $A_T>1$, multiply dilute times in the formula.
2. The protein concentrate of the sample needs to be determined by yourself and our PC0020 BCA Protein Assay Kit is recommended.
3. It is recommended to use the sample protein concentration to calculate the enzyme activity. If the sample fresh weight is used to calculate, the enzyme activity of cytoplasmic extract needs to be measured, and the sum of supernatant and precipitation enzyme activity is the total enzyme activity.
4. The reagent in this kit is enough to complete 25 tube reaction.
5. It is recommended to use the sample protein concentration to calculate the enzyme activity. If the
   sample fresh weight is used to calculate, the enzyme activity of cytoplasmic extract needs to be measured, and the sum of supernatant and precipitation enzyme activity is the total enzyme activity.
6. Attachment: calculation formula of sample weight: (the number of sample tests is 100T/48S)

1) Supernatant:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme generates 1 nmol of inorganic phosphorus per minute every gram tissue

ComplexV Activity(U/g)=)=$\Delta A1 \div A_S \times C \times Vrv \times 1000 \div (W \div Ve \times Vs) \div T = 20 \times \Delta A1 \div A_S \div W$

ΔA1: Supernatant absorbance; C:Standard solution concentration, 0.25μmol/mL. Vrv: Enzymatic reaction volume,0.12 mL;Ve: Extract solution volume,1 mL;Vs: Sample volume (mL), 0.05 mL;T: Reaction time (min), 30 minutes;W: Sample weight, g.

2) Sediment:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme generates 1

nmol of inorganic phosphorus per minute every gram tissue

ComplexV Activity(U/g)=ΔA2÷$A_S$×C×Vrv×1000÷(W÷Ve×Vs)÷T=12×ΔA2÷$A_S$÷W

ΔA2: Sediment absorbance; C:Standard solution concentration, 0.25μmol/mL. Vrv: Enzymatic reaction volume,0.12 mL; Ve: Sediment resuspended volume,0.6 mL; Vs: Sample volume (mL), 0.05 mL; T: Reaction time (min), 30 minutes; W: Sample weight, g.

3) Total activity is the sum of Complex IV activity in supernatant and sediment.

Complex V (U/g)=20×ΔA1÷$A_S$÷W+12×ΔA2÷$A_S$÷W

**Experimental example:**

1. Take 0.1g of rabbit kidney for sample treatment. After diluting the supernatant and sediment for 4 times, and operate according to the determination steps. Using 96 well flat-bottom plate, we measured ΔAs=As-Ab=0.379-0.047=0.332， Supernatant:ΔA1=$A_T$-Ac=0.679-0.119=0.560，Sediment:ΔA2=$A_T$-Ac=0.773-0.052=0.721.

   The activity of complex I in the supernatant (U/g mass) =20×ΔA1÷As÷W×4(Dilution ratio)=1349.4U/g mass

   The activity of complex I in the precipitation (U/g mass) = 12×ΔA2÷$A_S$÷W×4(Dilution ratio)=1042.4U/g mass

   Then complex I (U/g mass) =20×ΔA1÷$A_S$÷W+12×ΔA2÷$A_S$÷W=2391.8U/g mass.

2. Take 0.1g of holly for sample treatment, the supernatant and precipitation are diluted 4 times, according to the determination steps. Using 96 well flat-bottom plate, we measured ΔAs=As-Ab=0.379-0.047=0.332， Supernatant:ΔA1=$A_T$-Ac=0.535-0.320=0.215，Sediment:ΔA2=$A_T$-Ac=0.357-0.089=0.268.

   The activity of complex I in the supernatant (U/g mass) =20×ΔA1÷As÷W×4(Dilution ratio)=259.04 U/g mass

   The activity of complex I in the precipitation (U/g mass) = 12×ΔA2÷$A_S$÷W×4(Dilution ratio)=193.73 U/g mass

   Then complex I (U/g mass) =20×ΔA1÷$A_S$÷W+12×ΔA2÷$A_S$÷W=452.77U/g mass.

**Recent Products Citations：**

[1] Qiuli OuYang,Nengguo Tao,Miaoling Zhang. A Damaged Oxidative Phosphorylation Mechanism Is Involved in the Antifungal Activity of Citral against Penicillium digitatum. Frontier in Immunology. February 2018;(IF4.259)

[2] Wang M, Zhang Y, Xu M, et al. Roles of TRPA1 and TRPV1 in cigarette smoke-induced airway epithelial cell injury model[J].Free Radical Biology and Medicine,2019,134:229-238.

**Related Poducts:**

BC0510/BC0515 Mitochondrial Respiratory Chain Complex I Activity Assay Kit

BC3230/BC3235 Electron transport chain Complex II Activity Assay Kit

BC3240/BC3245 Electron transport chain Complex III Activity Assay Kit



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