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Mitochondrial Respiratory Chain Complex IV Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0274

Size:50T/48S

Components:

Extract solution: Liquid 80 mL×1. Storage at 4℃ .

Reagent I: Liquid 21 mL×1. Storage at 4℃ .

Reagent II: Powder×2. Storage at -20℃ .

Reagent III: Powder×2. Storage at 4℃ .

Working solution: Before use, transfer Reagent II and Reagent III to Reagent I for mixing and dissolution.

Product Description:

Mitochondrial Respiratory Chain Complex IV also knows as cytochrome c oxidase, is a common component of the main and branch of mitochondrial respiratory electron transport chain, and finally transfer electrons to oxygen to generate water.

Reduced cytochrome C has characteristic absorption peak at 550 nm, mitochondrial complex Ⅳ catalyzes the formation of oxidized cytochrome C from reduced cytochrome C. The enzyme activity of Complex IV can be calculated by detecting the decrease rate of reduced cytochrome C at 550 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, desk centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, acetone, ice and distilled water.

Procedure:

I. Complex extraction:

1) Collecting 0.1 g of tissue or 5 million cells, add 1 mL of Extract solution, grinding on ice with mortar/homogenizer. Centrifuge at 600 ×g for 10 minutes at 4℃ .

2) Take the supernatant to another tube and centrifuge at 11000 ×g for 15 minutes at 4℃ .

3) The supernatant can use to detect Complex Ⅳ that leaking from mitochondria, which shows the effect of mitochondrial extraction.

4) Add 400 μL of Extract solution to the sediment, splitting with ultrasonic (power 20%, work time 5s, interval 10s, repeat 15 times), used to detect the enzyme activity of Complex Ⅳ and protein content.

II. Determination procedure:

1) Preheat spectrophotometer for 30 minutes, adjust the wavelength to 550 nm, set zero with distilled water.

2) Preheat working solution at 37℃(mammal cell) or 25℃(other species) for 15 minutes. Unused reagent

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can be stored for one week at 4℃ .

3) Add the following reagents in 1 mL glass cuvette:

Reagent	Test tube (T)	Blank tube (B)
Sample (μL)	40	-
Distilled water	-	40
Working solution (μL)	800	800
Mix thoroughly and timing, detect the absorbance of initial and final reaction at 550 nm, record as A1(0s) and A2(1min) respectively. ΔA(T)=A2(T)-A1(T), ΔA(B)=A2(B)-A1(B).		

III. Calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1nmol of reduced cytochrome C per minute every milligram tissue protein.

Complex Ⅳ Activity(U/mg prot)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(Vs\times Cpr)\div T$ =1099×ΔA÷Cpr

ε: Cytochrome C molar extinction coefficient, 1.91×10^4 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume, 8.4×10^{-4} L;

Vs: Sample volume (mL), 0.04 mL;

Cpr: Sample protein concentration (mg/mL); The protein concentrate of the sample needs to be determined by yourself and our PC0020 BCA Protein Assay Kit is recommended;

T: Reaction time (min), 1 minute;

Note:

1. Take two or three different samples for prediction before test. Dilute supernatant with distilled water if the ΔA>0.2, multiply dilute times in the formular. While, increase the sample volume if ΔA is low.
2. Since the extract contains a relatively high concentration of protein, it is necessary to subtract the protein content of the extract itself when determining the protein concentration of the sample.
3. The reagent in this kit is enough to complete 50 tube reaction.
4. Attachment: calculation formula of sample weight: (the number of test samples is 50T/24S)

1) Supernatant:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1nmol of reduced cytochrome C per minute every gram of tissue.

Complex Ⅳ Activity(U/g)=$[\Delta A1\times Vrv\div(\varepsilon\times d)\times 10^9]\div(W\div Ve\times Vs)\div T$ =1099×ΔA1÷W

ΔA1: Supernatant absorbance;

Vrv: Total reaction volume,8.4×10^{-4} L;

ε: Cytochrome C molar extinction coefficient, 1.91×10^4 L/mol/cm;

d: Light path of cuvette, 1 cm;

Ve: Extract solution volume, 1 mL;

Vs: Sample volume (mL), 0.04 mL;

T: Reaction time (min), 1 minute;

W: Sample weight, g.

2) Sediment:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1nmol of reduced cytochrome C per minute every gram of tissue.

Complex Ⅳ Activity(U/g)=$[\Delta A2\times Vrv\div(\varepsilon\times d)\times 10^9]\div(W\div Ve\times Vs)\div T=440\times\Delta A2\div W$

ΔA2: Sediment absorbance;

Vrv: Total reaction volume, 8.4×10^{-4} L;

ε: Cytochrome C molar extinction coefficient,1.91×10^4 L/mol/cm;

d: Light path of cuvette, 1 cm;

Ve: Sediment resuspended volume, 0.4 mL;

Vs: Sample volume (mL), 0.04 mL;

T: Reaction time (min), 1 minute;

W: Sample weight, g.

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

Complex Ⅳ(U/g)=1099×ΔA1÷W +440×ΔA2÷W.

Experimental example:

1. 0. 1g of rabbit liver is taken for sample processing, and the operation is performed according to the determination steps. Using micro glass cuvette, supernatant: ΔA2 = $A1_B-A2_B$ = 0.79-0.781=0.009, ΔA1 = $A1_T-A2_T$ = 0.822-0.792=0.03, ΔA supernatant = ΔA1-Δ A2 = 0.03-0.009=0.021, precipitation: ΔA1 = A1T-A2T =0.992-0.792=0.2, ΔA precipitation = ΔA1 - ΔA2 = ΔA1-ΔA2= 0.2-0.009=0. 191

The activity of complex IV in supernatant (U/g mass) = 1099×ΔA supernatant ÷ W = 1099×0.021÷0. 1 =230.79 U/g mass

The activity of complex IV in the precipitation (U/g mass) = 440×ΔA precipitation÷ W = 440×0. 191÷0. 1 =840.4 U/g mass

Then the total activity of complex IV (U/g mass) = 1099×ΔA supernatant ÷ W + 440×ΔA precipitation ÷W

=1099×0.021÷0. 1+440×0. 191÷0. 1=1071. 19 U/g mass.

Recent Product 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] Huazhang Zhu,Weizhen Zhang,Yingying Zhao,et al. GSK3β-mediated tau hyperphosphorylation triggers diabetic retinal neurodegeneration by disrupting synaptic and mitochondrial functions. Molecular Neurodegeneration. November 2018;(IF8.274)

[3] 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.

[4] Bao Z, Xu X, Chao H, et al. ERK/Nrf2/HO- 1 pathway-mediated mitophagy alleviates traumatic brain injury-induced intestinal mucosa damage and epithelial barrier dysfunction[J]. 2017.

[5] Li N, Qin S, Xie L, et al. Elevated Serum Potassium Concentration Alleviates Cerebral Ischemia-Reperfusion Injury via Mitochondrial Preservation[J]. Cellular Physiology and Biochemistry, 2018, 48(4): 1664- 1674.

References:

[1] Willis J H, Capaldi R A, Huigsloot M, et al. Isolated deficiencies of OXPHOS complexes I and IV are identified accurately and quickly by simple enzyme activity immunocapture assays[J]. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2009, 1787(5): 533-538.

Related Products:

AK0544/AK0543	Electron Transport Chain Complex I Activity Assay Kit
AK0368/AK0367	Electron transport chain Complex Ⅱ Activity Assay Kit
AK0366/AK0365	Electron transport chain Complex Ⅲ Activity Assay Kit
AK0263/AK0021	Electron transport chain Complex Ⅴ Activity Assay Kit