Mitochondrial Respiratory Chain Complex IV Activity Assay Kit

(Cytochrome C Oxidase Activity)

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: BC0945

Size:100T/96S

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 75 mL×2	2-8℃
Reagent I	Liquid 33 mL×1	2-8℃
Reagent II	Powder×2	-20℃
Reagent III	Powder×2	2-8℃

Solution Preparation:

1. Reagent II: The reagent is placed in the glass bottle inside the bottle. Add 13.5 mL Reagent I to one Reagent II before use. It could be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.
2. Reagent III: The reagent is placed in the glass bottle inside the bottle. Add 2 mL Reagent I to one Reagent III before use. It could be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.
3. Working solution: Add 0.5 mL Reagent III to 4.5 mL Reagent II (about 25T) before use. Or prepared according to sample numbers.

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 a 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/microplate reader, water bath/constant temperature incubator, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, 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 used 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 200W, 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 microplate reader/spectrophotometer for 30 minutes, adjust the wavelength to 550 nm, set spectrophotometer to zero with distilled water.

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

3) Add the following reagents in micro glass cuvette/96 well flat-bottom plate:

Reagent (μL)	Test tube (T)	Blank tube (B)
Sample	10	-
Distilled water	-	10
Working solution	200	200
Mix thoroughly and timing, detect the absorbance of initial and final reaction at 550 nm, record as A1(10s) and A2(1min10s) respectively. ΔA(T)=A1(T)-A2(T), ΔA(B)=A1(B)-A2(B). ΔA=ΔA(T)- ΔA(B). Blank tube needs to test once or twice.		

III. Calculation:

A. micro glass cuvette

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)=[ΔA×Vrv÷(ε×d)×10^9]÷(Vs×Cpr)÷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, 2.1×10^{-4} L;

Vs: Sample volume, 0.01 mL;

Cpr: Sample protein concentration, mg/mL;

T: Reaction time (min), 1 minute;

10^9: Unit conversion factor, 1 mol=10^9 nmol.

B. 96 well flat-bottom plate

Change the d-1cm in the above formula to d-0.6cm (the optical diameter of cuvette) for calculation.

Note:

1. Take two or three different samples for prediction before test. Dilute supernatant with distilled water if A1>1 or ΔA>0.4, multiply dilute times in the formular. 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 100T/48S)

A. 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 mass)=[ΔA1×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =1099×ΔA1÷W

ΔA1: Supernatant absorbance;

Vrv: Total reaction volume, 2.1×10^{-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), 0.01 mL;

T: Reaction time (min), 1 minute;

W: Sample weight, g;

10^9: Unit conversion factor, 1 mol=10^9 nmol.

B. 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 mass)=[ΔA2×Vrv÷(ε×d)×10^9]÷(W÷Ve×Vs)÷T =440×ΔA2÷W

ΔA2: Sediment absorbance;

Vrv: Total reaction volume, 2.1×10^{-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), 0.01 mL;

T: Reaction time (min), 1 minute;

W: Sample weight, g;

10^9: Unit conversion factor, 1 mol=10^9 nmol.

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

Complex IV Activity(U/g mass)=1099×ΔA1÷W +440×ΔA2÷W.

D. Detect by 96 well UV plate

Change the d-1cm in the above formula to d-0.6cm (the optical diameter of 96 well UV plate) for calculation.

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: $\Delta A2 = A1_B\text{-}A2_B$ = 0.7713-0.7669 = 0.0044, $\Delta A1 = A1_T\text{-}A2_T$ = 0.7985-0.7754 = 0.0231, ΔA supernatant = ΔA1-ΔA2 = 0.0231-0.0044 = 0.0187, precipitation: ΔA1 = A1T-A2T = 0.8843-0.7415 = 0.1428, ΔA precipitation = ΔA1 - ΔA2 = 0.1428-0.0044 = 0.1384

The activity of complex IV in supernatant (U/g mass) = 1099×0.0187 ÷ 0.1 = 205.513 U/g mass

The activity of complex IV in the precipitation (U/g mass) = 440×0.1384 ÷0.1 = 608.96 U/g mass

The total activity of complex IV (U/g mass) =1099×0.0187÷0.1+440×0.1384÷0.1=814.473 U/g mass.

Recent Product Citations:

[1] Zhou N, Qi H, Liu J, Zhang G, Liu J, Liu N, Zhu M, Zhao X, Song C, Zhou Z, Gong J, Li R, Bai X, Jin Y, Song Y, Yin Y. Deubiquitinase OTUD3 regulates metabolism homeostasis in response to nutritional stresses. Cell Metab. 2022 Jul 5;34(7):1023-1041.e8. doi: 10.1016/j.cmet.2022.05.005. Epub 2022 Jun 7. PMID: 35675826.

[2] Li C, Wu B, Li Y, Chen J, Ye Z, Tian X, Wang J, Xu X, Pan S, Zheng Y, Cai X, Jiang L, Zhao M. Amino acid catabolism regulates hematopoietic stem cell proteostasis via a GCN2-eIF2 axis. Cell Stem Cell. 2022 Jul 7;29(7):1119-1134.e7. doi: 10.1016/j.stem.2022.06.004. PMID: 35803229.

[3] Zhou Y, Tang J, Lan J, Zhang Y, Wang H, Chen Q, Kang Y, Sun Y, Feng X, Wu L, Jin H, Chen S, Peng Y. Honokiol alleviated neurodegeneration by reducing oxidative stress and improving mitochondrial function in mutant SOD1 cellular and mouse models of amyotrophic lateral sclerosis. Acta Pharm Sin B. 2023 Feb;13(2):577-597. doi: 10.1016/j.apsb.2022.07.019. Epub 2022 Aug 10. PMID: 36873166; PMCID: PMC9979194.

[4] Xin J, Zhu B, Wang H, Zhang Y, Sun N, Cao X, Zheng L, Zhou Y, Fang J, Jing B, Pan K, Zeng Y, Zeng D, Li F, Xia Y, Xu P, Ni X. Prolonged fluoride exposure induces spatial-memory deficit and hippocampal dysfunction by inhibiting small heat shock protein 22 in mice. J Hazard Mater. 2023 Aug 15;456:131595. doi: 10.1016/j.jhazmat.2023.131595. Epub 2023 May 7. PMID: 37224709.

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:

BC0510/BC0515	Electron Transport 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
BC1440/BC1445	Electron transport chain Complex V Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com