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CheKine™ Micro Mitochondrial Complex Ⅳ Activity Assay Kit

Cat #: KTB1880 Size: 48 T/48 S 96 T/96 S

	Micro Mitochondrial Complex Ⅳ Activity Assay Kit		
REF	**Cat #:** KTB1880	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Mitochondrial respiratory chain complex Ⅳ, also known as cytochrome C oxidase, is also a common component of the main and branch pathways of the mitochondrial respiratory chain. It is responsible for catalyzing the oxidation of reduced cytochrome C, and ultimately transferring electrons to oxygen to produce water. CheKine™ Micro Mitochondrial Complex Ⅳ Activity Assay Kit provides a convenient tool for detection of Mitochondrial complex Ⅳ Activity. The principle is Reduced cytochrome C has characteristic light absorption at 550 nm. Mitochondrial respiratory chain complex IV could catalyze reduced cytochrome C to oxidized cytochrome C. Therefore, the decreasing rate of light absorption at 550 nm can reflect the enzyme activity of mitochondrial respiratory chain complex IV. It can be used to determine animal, plant tissues and cell samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	60 mL×2	4℃
Reagent Ⅱ	12 mL	24mL	4℃
Reagent Ⅲ	1 mL	2 mL	4℃, protected from light
Reagent Ⅳ	12 mL	24 mL	4℃
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅵ	Powder×1 vial	Powder×1 vial	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 550 nm
- Incubator, ice maker, refrigerated centrifuge

- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer or mortar

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Working Solution: Transfer the Reagent Ⅴ and Reagent Ⅵ respectively to the tube of Reagent Ⅳ, and mixing well before use. Then incubated the mixture at 37℃ for 5 min if the detected samples are from mammalian, or incubated at 25℃ for 5 min if the samples are from another species. The remaining reagent can be stored at -20℃ and protected from light for a week after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended to ensure enzyme activity.

Extraction of Mitochondrial Respiratory Chain Complex Ⅳ:

1. Accurately weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Reagent Ⅰ and 10 μL Reagent Ⅲ, homogenize or mortar on ice.
2. Centrifuge the homogenate with 600 g for 5 min at 4℃, collect the supernatant to a new centrifuge tube and discard the pellet.
3. Centrifuge the supernatant again with 11,000 g for 10 min at 4℃. The pellet is the extracted mitochondria, which could be used to do step 5.
4. (Optional) The supernatant is cytoplasmic extract, which can be used as sample to determine mitochondrial respiratory chain complex Ⅳ leaking from mitochondria to judge the effect of mitochondrial extraction.
5. Add 200 μL Reagent Ⅱ and 2 μL Reagent Ⅲ to the pellet, resuspend the pellet sufficiently, and use it to detect the activity of mitochondrial respiratory chain complex Ⅳ in the next step.

Assay Procedure

1. Preheated the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 550 nm, visible spectrophotometer was returned to zero with deionized water.
2. Successively add 200 μL Working Solution and 10 μL sample into the 96-well plate or microglass cuvette, mix well. Immediately read the initial absorbance value (0 min) at 550 nm as A_1, and then read again after 1 min as A_2. Finally calculate $\Delta A=A_1-A_2$.

Note: 1. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If ΔA is too high (above 1.0), the samples should be dilute with Reagent Ⅱ and then measured again . Pay attention to multiply by the dilution factor when calculating the result. If ΔA is too small, the sensitivity can be improved by increasing the sample volume added. If ΔA is negative, it means that complex Ⅳ is not contained in the sample or has been degraded. 2. The mitochondrial respiratory chain kit is based on the principle of enzyme kinetics, and the reaction is relatively fast, and after the reaction tends to balance, the reversible reaction may have a negative reaction. The suggestions are as follows: (1) The number of sample groups: about 2-3, the enzymatic reaction speed is fast, and it is an enzymatic reaction, it is necessary to grasp the starting time point and the time point after the reaction; (2) The instrument is preheated in advance, and the sample addition can be arranged next to the enzyme marker, and the sample is added directly after mixing; (3) If ΔA is too small, the sample size (tissue weight or cell number) can be increased, or the amount of extraction liquid can be reduced; (4) The samples should be extracted as fresh as possible. If they cannot be used immediately, the whole cells or packaged tissues should be stored at -80℃ for use within one month. (5) Preparation of Working Solution before use. 3. The samples extracted by this kit can also be used for the determination of KTB1850, KTB1860, KTB1870 and KTB1890.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. 96-well plates calculation formula as below

(1) Calculated by fresh weight of samples

Unit definition: One enzyme activity unit defines as the consumption of 1 nmol reduced cytochrome C in 1 g tissue per min in the reaction system.

Calculate the activity of Complex Ⅳ in the supernatant:

The activity of supernatant Complex Ⅳ(U/g fresh weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extracttion}\times V_{Sample})\div T$**=2,221×$\Delta A_{Supernatant}\div W$**

Calculate the activity of Complex Ⅳ of the mitochondrial pellet:

The activity of pellet Complex Ⅳ(U/g fresh weight)=$[\Delta A_{Pellet}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Resuspended}\times V_{Sample})\div T$**=444×$\Delta A_{Pellet}\div W$**

Calculate the total activity of Complex Ⅳ in sample:

The total activity of Complex Ⅳ in sample is the sum of the supernatant and pellet.

Total activity (U/g fresh weight)**=2,221×$\Delta A_{Supernatant}\div W$+ 444×$\Delta A_{Pellet}\div W$**

(2) Calculated by cell density

Unit definition: Every 10,000 cells consume 1 nmol reduced cytochrome C per minute that is defined as one enzyme activity.

The activity of Complex Ⅳ(U/10^4 cells)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Resuspend}\times 500)\div T$**=0.888×ΔA**

Where: V_{Total}: total reaction volume, 2.1×10^{-4} L; ε: reduced cytochrome C molar extinction coefficient, 19.1×10^3 mol/L/cm; d: 96-well plate diameter, 0.5 cm; 10^9: Unit conversion factor, 1 mol=10^9 nmol; V_{Sample}: sample volume added, 0.01 mL; T: reaction time, 1 min; $\Delta A_{Supernatant}$: determination value of the supernatant; W: sample weight, g; $V_{Extracttion}$: sample extract volume, 1.01 mL; ΔA_{Pellet}: determination value of pellet; $V_{Resuspended}$: volume of the resuspend pellet 0.202 mL; 500: total number of cells, 5×10^6.

2. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation

Typical Data

Examples:

1. Test 0.1g mouse brain tissue, prepared the sample following the above protocol and measured with the 96-well microplate:

$\Delta A_{Supernatant}=A_1-A_2=0.4568-0.4446=0.0122$, $\Delta A_{Pellet}=A_1-A_2=0.4373-0.4188=0.0185$

2. Calculated by fresh weight of samples,

Complex Ⅳ activity of the supernatant (U/g fresh weight)=$2,221\times\Delta A_{Supernatant}\div W=2,221\times0.0122\div0.1=270.962$ U/g

Complex Ⅳ activity of the pellet (U/g fresh weight) =$444\times\Delta A_{Pellet}\div W=444\times0.0185\div0.1=82.14$ U/g

The total Complex Ⅳ activity (U/g fresh weight)=$2,221\times\Delta A_{Supernatant}\div W+444\times\Delta A_{Pellet}\div W=270.962+82.14=353.102$ U/g

Recommended Products

Catalog No.	Product Name
KTB1850	CheKine™ Micro Mitochondrial Complex Ⅰ Activity Assay Kit
KTB1860	CheKine™ Micro Mitochondrial Complex Ⅱ Activity Assay Kit
KTB1870	CheKine™ Micro Mitochondrial Complex Ⅲ Activity Assay Kit
KTB1890	CheKine™ Micro Mitochondrial Complex Ⅴ Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.