CheKine™ Micro Mitochondrial Complex Ⅴ Activity Assay Kit

Cat #: KTB1890 Size: 48 T/96 T

	Micro Mitochondrial Complex Ⅴ Activity Assay Kit		
REF	**Cat #:** KTB1890	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 Ⅴ, also known as F1F0-ATP synthase, is widely present in the mitochondria of animals, plants, microorganisms and cultured cells. Complex Ⅴ consists of two subunits, F1 and F0. It could use the electrochemical gradient of protons produced by the respiratory chain to catalyze the synthesis of ATP, and also can hydrolyze ATP in a reversible process. In addition, complex Ⅴ also exists in chloroplasts, heterotrophic and photosynthetic. Complex Ⅴ is the key enzyme for mitochondrial oxidative phosphorylation and chloroplast photosynthetic phosphorylation to synthesize ATP. CheKine™ Micro Mitochondrial Complex Ⅴ Activity Assay Kit provides a convenient tool for detection of Mitochondrial Complex Ⅴ Activity. The principle is Complex Ⅴ hydrolyzes ATP to produce ADP and Pi. The activity of complex Ⅴ is determined by measuring Pi increase rate. It can be used to determine animal, plant tissue and cell samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4℃
Reagent Ⅱ	40 mL	80 mL	4℃
Reagent Ⅲ	1 mL	2 mL	4℃, protected from light
Reagent Ⅳ	1	1	-20℃, protected from light
Reagent Ⅴ	4 mL	8 mL	4℃
Reagent Ⅵ	1	1	4℃, protected from light
Reagent Ⅶ	1	1	4℃, protected from light
Reagent Ⅷ	1	1	4℃, protected from light
Reagent Ⅸ	5 mL	10 mL	RT

Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer or mortar (for tissue samples)

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℃.

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

ReagentⅣ: Add 1 mL deionized water for 48 T and 2 mL deionized water for 96 T to fully dissolve before use. The rest of reagent can also be stored at -20℃ and protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

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

ReagentⅥ: Add 2 mL deionized water for 48 T and 4 mL deionized water for 96 T to fully dissolve before use. The rest of reagent can also be stored at -20℃ and protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

ReagentⅦ: Add 5 mL deionized water for 48 T and 10 mL deionized water for 96 T to fully dissolve before use. The rest of reagent can also be stored at -20℃ and protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

ReagentⅧ: Add 5 mL deionized water for 48 T and 10 mL deionized water for 96 T to fully dissolve before use. The rest of reagent can also be stored at -20℃ and protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

ReagentⅨ: Ready to use as supplied.

Prepare the phosphorus quantitative reagent: Prepare according to the ratio as deionized water: ReagentⅦ: ReagentⅧ: ReagentⅨ=2:1:1:1, the color of the prepared phosphorus quantitative reagent should be yellow. The reagent maybe invalid if there is no color, or maybe phosphorus pollution if the color is blue. So, please prepare it freshly depend on your need.

Note: It would be better to use new glassware or disposable plastic ware, please avoid the phosphorus pollution.

Setting of Standard curves: Before use, dilute 10 mM standard with deionized water to 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 mM standard solution as shown in the table below.

Num.	Volume of Standard	Volume of deionized water (μL)	Concentration (mM)
Std.1	100 μL 10 mM	900	1
Std.2	100 μL of Std.1 (1 mM)	100	0.5
Std.3	100 μL of Std.2 (0.5 mM)	100	0.25
Std.4	100 μL of Std.3 (0.25 mM)	100	0.125
Std.5	100 μL of Std.4 (0.125 mM)	100	0.0625
Std.6	100 μL of Std.5 (0.0625 mM)	100	0.0313
Std.7	100 μL of Std.6 (0.0313 mM)	100	0.0156

Sample Preparation

Note: Fresh samples are recommended to ensure enzyme activity.

Extraction of Mitochondrial Respiratory Chain Complex Ⅴ:

1. Accurately weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Reagent Ⅰ and 10 μL ReagentⅢ, 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 Ⅴ leaking from mitochondria to judge the effect of mitochondrial extraction.
5. Add 800 μL Reagent Ⅱ and 8 μL Reagent Ⅲ to the pellet, resuspend the pellet sufficiently, and use it to detect the activity of mitochondrial respiratory chain complex Ⅴ in the next step.

Assay Procedure

1. Preheated the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.
2. Enzymatic reaction (add the following reagents respectively into each EP tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
ReagentⅣ	0	0	10	10
Reagent Ⅴ	0	0	40	40
Sample	0	0	50	0
Mix well, incubate in 37°C for mammalian sample, or incubate in 25°C for other species, 30 min				
Reagent Ⅵ	0	0	20	20
Sample	0	0	0	50

3. Mix well, Centrifuge at 4,000 g for 10 min at room temperature, collect the supernatant.
4. Phosphorus quantitative, add the following reagent into the 96-well plate or microglass cuvette carefully.

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Supernatant	0	0	40	40
Standards	0	40	0	0
Deionized Water	40	0	0	0
Phosphorus Quantitative Reagent	200	200	200	200

5. Mix well, standing at room temperature for 10 min. Then reading the absorbance at 660 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Control}$. Blank Tube only need to measure 1 time.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If the absorbance values is too high (> 1.5). The samples should be diluted with Reagent Ⅱ and then measured again. Pay attention to multiply by the dilution factor when calculating the result. If ΔA_{Test} is too small, the sensitivity can be improved by increasing the sample volume added. If ΔA_{Test} is negative, it means that complex Ⅴ is not contained in the sample or has been degraded. Reagent Ⅲ has certain toxicity, and Reagent Ⅸ is corrosive. Please take protective measures when operating.

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. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculate the content of inorganic phosphorus (Pi)

Substitute the ΔA_{Test} into the equation to obtain the y value (mM).

3. Calculate complex Ⅴ activity

(1) Calculated by fresh weight of samples

Unit definition: one enzyme activity unit defines as 1 nmol of inorganic phosphorus produced by per g tissue per minute in the reaction system.

Calculate the activity of Complex Ⅴ in the supernatant:

The activity of Complex Ⅴ (U/g fresh weight)=$(y_{Supernatant}\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Extraction}\times W)\div T$**=80.8×$y_{Supernatant}$÷W**

Calculate the activity of Complex Ⅴ of the mitochondrial pellet:

The activity of Complex Ⅴ (U/g fresh weight)=$(y_{Pellet}\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Respended}\times W)\div T$**=64.64×$y_{Pellet}$÷W**

Calculate the total activity of Complex Ⅴ in the sample:

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

Total activity (U/g fresh weight)**=80.8×$y_{Supernatant}$÷W+ 64.64×y_{Pellet}÷W**

(2) Calculated by cell density

Unit definition: one enzyme activity unit defines as 1 nmol of inorganic phosphorus produced by per 10,000 cells per minute in the reaction system.

The activity of Complex Ⅴ (U/10^4 cells)=$(y\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Respended}\times 500)\div T$**=0.129×y**

Where: $V_{Enzymatic\ Reaction}$: total Enzymatic reaction volume, 1.2×10^{-4} L; 10^6: Unit conversion factor, 1 mmol=10^6 nmol; V_{Sample}: sample volume added, 0.05 mL; T: reaction time, 30 min; $V_{Extraction}$: sample extract volume, 1.01 mL; W:sample weight, g; $V_{Resuspend}$: Volume of the resuspend pellet ,0.808 mL; 500: Total number of cells, 5 million.

Typical Data

Typical standard curve

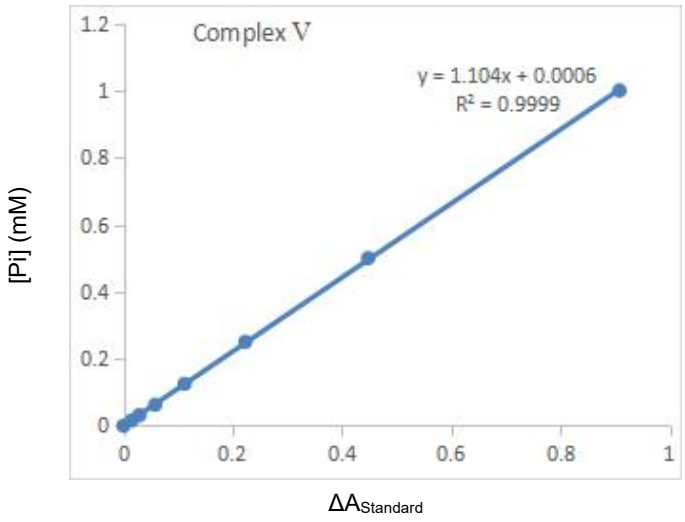

Figure1. Standard curve of the activity of complex Ⅴ in 96-well plate assay–data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Examples:

1. Test 0.1 g Epipremnum aureum tissue, prepared the sample following the above protocol and measured with the 96-well plate: $\Delta A_{Supernatant}=A_{Test}-A_{Control}$ =0.404-0.267=0.137, $\Delta A_{Pellet}=A_{Test}-A_{Control}$ =0.355-0.142=0.213

Substitute the ΔA_{Test} into the equation to obtain the $y_{Supernatant}$=0.1518, y_{Pellet}=0.2358

2. Calculated by fresh weight of samples:

Complex Ⅴ activity of the supernatant (U/g fresh weight)=80.8×$y_{Supernatant}$÷W=80.8×0.1518÷0.1=122.654 U/g

Complex Ⅴ activity of the pellet (U/g fresh weight)=64.64×y_{Pellet}÷W=64.64×0.2358÷0.1=152.421 U/g

The total complex Ⅴ activity (U/g fresh weight)=122.654+152.421=275.075 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
KTB1880	CheKine™ Micro Mitochondrial Complex Ⅳ Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.