CheKine™ Plus Micro Mitochondrial Complex Ⅴ Activity Assay Kit

Cat #: KTB1891 Size: 48 T/48 S 96 T/96 S

	Plus Micro Mitochondrial Complex Ⅴ Activity Assay Kit		
REF	**Cat #:** KTB1891	LOT	**Lot #:** Refer to product label
	Detection range: 0.0313-2 mM		**Sensitivity:** 0.0313 mM
	Applicable samples: Animal and Plant Tissues, Cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Note: The detection range and sensitivity here refer to standard, which need to be converted to Complex Ⅴ activity based on sample conditions.

Assay Principle

Mitochondrial respiratory chain complex Ⅴ (EC 7.1.2.2), 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™ Plus 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.

This kit is specifically optimized for low-activity sample scenarios by enhancing the enzyme-coupled reaction system, significantly improving detection performance across low concentration ranges. It simultaneously reduces component complexity and shortens reaction time, further simplifying operational procedures. The kit is compatible with high-throughput batch sample testing and comprehensively addresses the detection challenges of mitochondrial respiratory chain complexes.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	60 mL×2	4℃
Reagent Ⅱ	48 mL	96 mL	4℃
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅳ	4.8 mL	9.6 mL	4℃
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	4℃, protected from light

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Reagent Ⅵ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅶ	20 mL	40 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃, 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 660 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. Placed on ice during the experiment. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Placed on ice during the experiment. Store at 4℃.

Working Reagent Ⅲ: Prepared before use. Add 1.2 mL deionized water for 48 T and 2.4 mL deionized water for 96 T to fully dissolve. The prepared Working Reagent Ⅲ should be stored at -20℃, protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

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

Working Reagent Ⅴ: Prepared before use. Add 2.4 mL deionized water for 48 T and 4.8 mL deionized water for 96 T to fully dissolve. The prepared Working Reagent Ⅴ should be stored at -20℃, protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

Working Reagent Ⅵ: Prepared before use. Add 5 mL deionized water for 48 T and 10 mL deionized water for 96 T to fully dissolve. The prepared Working Reagent Ⅵ should be stored at -20℃, protected from light after aliquoting for 1 month to avoid repeated freezing and thawing.

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

Standard: Ready to use as supplied. Equilibrate to room temperature before use. The concentration is 10 mM. Store at 4℃, protected from light.

Working Solution: Prepared before use. Prepare the Working Solution by adding 200 μL per well (Working Reagent Ⅵ 40 μL : Reagent Ⅶ 160 μL, with a mixing ratio of 1:4), and mix thoroughly. The prepared Working Solution should be stored at -20℃, protected from light after aliquoting for 3 days to avoid repeated freezing and thawing. The color of the Working Solution should be light yellow. The reagent maybe invalid if there is no color, or maybe phosphorus pollution if the color is blue.

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 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0313 mM standard solution as shown in the table below.

Num.	Volume of Standard	Volume of deionized water (μL)	Concentration (mM)
Std.1	100 μL of 10 mM	400	2
Std.2	200 μL of Std.1 (2 mM)	200	1
Std.3	200 μL of Std.2 (1 mM)	200	0.5
Std.4	200 μL of Std.3 (0.5 mM)	200	0.25
Std.5	200 μL of Std.4 (0.25 mM)	200	0.125

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Std.6	200 μL of Std.5 (0.125 mM)	200	0.0625
Std.7	200 μL of Std.6 (0.0625 mM)	200	0.0313
Blank	0	200	0

Sample Preparation

Note: Fresh samples are recommended to ensure enzyme activity. The samples should be extracted as fresh as possible. If immediate use is not required, store whole cells or tissues at -80℃ for 1 month. After the extracted samples are resuspended with Reagent II, it should be detected as soon as possible. If testing cannot be done on the same day, store resuspension solution at -20℃ for 1 day. The samples extracted by this kit can also be used for the determination of KTB1851, KTB1861, KTB1871 and KTB1881 (for detailed instructions, refer to the Precautions).

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, homogenize or mortar on ice. **Manual homogenization is recommended. If a large number of samples are prepared at one time, mechanical homogenization (in an ice bath) can be used to improve efficiency. The recommended parameters are 40 Hz, homogenization for 20 s, with a 10 s interval, repeated 3 times (2-3 3 mm steel beads). Direct mechanical homogenization of plant samples may result in enzyme activity decrease. It is recommended to conduct a pre-experiment to evaluate based on your own equipment conditions or use manual homogenization. Cells may also be processed by ice bath ultrasonic disruption for 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s).**
2. Centrifuge the homogenate at 600 g for 5 min at 4℃, collect the supernatant to a new centrifuge tube and discard the pellet.
3. Centrifuge the supernatant again at 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 deter
5. mine mitochondrial respiratory chain complex Ⅴ leaking from mitochondria to judge the effect of mitochondrial extraction.
6. Add 800 μL of Reagent Ⅱ to the pellet, use a pipette to lift and gently shake the pellet, and thoroughly resuspend it (avoid using a vortex mixer to mix vigorously as it may damage the complex; for subsequent steps, a gentle mixing with the vortex mixer can be used).

Assay Procedure

1. Preheat 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). **This step is not required for blank tube and standard tube.**

Reagent	Test Tube (μL)	Control Tube (μL)
Reagent Ⅲ	10	10
Reagent Ⅳ	40	40
Sample	50	0
Mix well, incubate at 37℃ (mammal and mammalian cell samples), or 25℃ (other species) for 15 min		
Reagent Ⅴ	20	20
Sample	0	50

3. Mix well, Centrifuge at 6,000 g for 10 min at room temperature (25℃), collect the supernatant to new EP tubes for testing (The supernatant can be stored at -20℃ for 1 day.).

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4. Detection System Preparation: 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
Working Solution	200	200	200	200

5. Mix well, stand at room temperature for 10 min. Then read the absorbance at 660 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. Blank Well and Standard Well only need to be measured 1 time.

Note: 1. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If the ΔA_{Test} exceeds the $\Delta A_{Standard}$ of 2 mM, the supernatant obtained after centrifugation of the enzymatic reaction can be diluted with deionized water, and then measured again, the final result should be multiplied by the dilution factor. If ΔA_{Test} is less than 0.02, the volume of added supernatant can be increased appropriately, or the sample volume or sample quantity (e.g., tissue weight or cell count) can be increased before re-measurement. A negative ΔA_{Test} value indicates the absence of mitochondrial respiratory chain complex Ⅴ or its degradation.

2. If multiple samples are detected simultaneously, use a multi-channel pipette can increase the speed of sample addition.

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.

The activity of supernatant Complex Ⅴ (U/g)=$(y_{Supernatant}\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Extraction}\times W)\div T$**=$160\times y_{Supernatant}\div W$**

The activity of pellet Complex Ⅴ (U/g)=$(y_{Pellet}\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Resuspended}\times W)\div T$**=$128\times y_{Pellet}\div W$**

Total activity (U/g)**=$160\times y_{Supernatant}\div W+128\times y_{Pellet}\div W$**

(2) Calculated by cell density

Unit definition: one enzyme activity unit defines as 1 nmol of inorganic phosphorus produced by per 10^4 cells per minute in the reaction system.

The activity of supernatant Complex Ⅴ (U/10^4)=$(y_{Supernatant}\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Extraction}\times N)\div T$**=$160\times y_{Supernatant}\div N$**

The activity of pellet Complex Ⅴ (U/10^4)=$(y_{Pellet}\times V_{Enzymatic\ Reaction}\times 10^6)\div(V_{Sample}\div V_{Resuspended}\times N)\div T$**=$128\times y_{Pellet}\div N$**

Total activity (U/10^4)**=$160\times y_{Supernatant}\div N+128\times y_{Pellet}\div N$**

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, 15 min; $V_{Extraction}$: sample extract volume, 1 mL; W:sample weight, g; $V_{Resuspended}$: Volume of the resuspend pellet ,0.8 mL; N: total number of cells, use 10^4 as the unit (e.g., total number of cells: 5×10^6, N=500).

Precautions

1. Why does the supernatant appear clear and transparent during color development, while the pellet appears turbid?

Molybdenum blue is a nanoscale colloidal particle formed by the reduction of phosphomolybdic acid, belonging to a

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non-molecularly dispersed suspension (colloidal system). The sample pellet contains impurity particles such as mitochondrial membrane fragments, lipoproteins, and cell lysate particles, which serve as interfaces for heterogeneous nucleation, promoting rapid adsorption and aggregation of molybdenum blue particles into larger aggregates that exhibit strong light scattering and appear milky white and turbid. In contrast, the supernatant contains minimal impurities; the molybdenum blue particles lack nucleation sites and exist primarily in monodisperse or small aggregates with particle sizes significantly smaller than the visible light wavelength, resulting in weak light scattering and a clear, transparent solution.

2. For the same sample, all reagent kits (KTB1851, KTB1861, KTB1871, KTB1881, and KTB1891) can perform detection. How should this be operated?

(1) Reagent Ⅰ and Reagent Ⅱ in the KTB1851, KTB1861, KTB1871, KTB1881, and KTB1891 kits are interchangeable; Reagent Ⅰ and Reagent Ⅱ from any of these kits may be used to prepare samples.

(2) Accurately weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Reagent Ⅰ, homogenize or mortar on ice. **Manual homogenization is recommended. If a large number of samples are prepared at one time, mechanical homogenization (in an ice bath) can be used to improve efficiency. The recommended parameters are 40 Hz, homogenization for 20 s, with a 10 s interval, repeated 3 times (2-3 3 mm steel beads). Direct mechanical homogenization of plant samples may result in enzyme activity decrease. It is recommended to conduct a pre-experiment to evaluate based on your own equipment conditions or use manual homogenization. Cells may also be processed by ice bath ultrasonic disruption for 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s).**

(3) Centrifuge the homogenate at 600 g for 5 min at 4℃, collect the supernatant to a new centrifuge tube and discard the pellet.

(4) Centrifuge the supernatant again at 11,000 g for 10 min at 4℃. The pellet is the extracted mitochondria, which could be used to do step (6).

(5) (Optional) The supernatant is cytoplasmic extract, which can be used as sample to determine mitochondrial respiratory chain complex Ⅰ-Ⅴ leaking from mitochondria to judge the effect of mitochondrial extraction.

(6) Add 200 μL of Reagent Ⅱ to the pellet, use a pipette to lift and gently shake the pellet, and thoroughly resuspend it (avoid using a vortex mixer to mix vigorously as it may damage the complex; for subsequent steps, a gentle mixing with the vortex mixer can be used). After completing step 7, place the sample on ice for testing.

(7) For the detection of Complex Ⅰ, Complex Ⅱ, and Complex Ⅲ, samples should be diluted with Reagent Ⅱ according to the recommended dilution ratios specified in the instructions for KTB1851, KTB1861, and KTB1871, respectively. For Complex Ⅳ, samples must first be diluted 2-fold with Reagent Ⅱ before further dilution using Reagent Ⅱ according to the recommended ratio in the KTB1881 instructions. For Complex Ⅴ, samples should be diluted 4-fold with Reagent Ⅱ prior to direct use for detection.

Typical Data

Typical standard curve

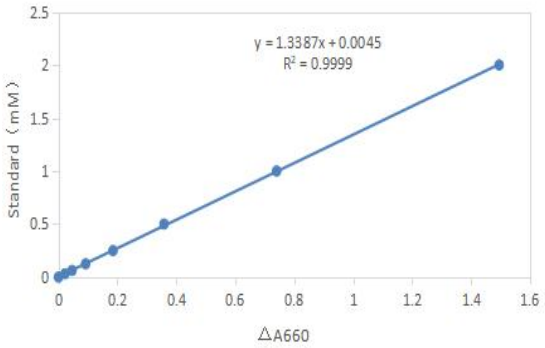


Figure1. Standard curve of the activity of complex Ⅴ in 96-well plate assay–data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Examples:

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1. Test 0.1 g Rabbit liver tissue, prepared the sample following the above protocol and measured with the 96-well plate:

$\Delta A_{Supernatant}=A_{Test}-A_{Control}$ =1.720-0.566=1.154, $\Delta A_{Pellet}=A_{Test}-A_{Control}$ =1.483-0.580=0.903

Substitute the $\Delta A_{Supernatant}$ and ΔA_{Pellet} into the equation to obtain the $y_{Supernatant}$=1.549, y_{Pellet}=1.213

2. Calculated by fresh weight of samples:

Complex Ⅴ activity of the supernatant (U/g)=$160\times y_{Supernatant}\div W=160\times1.549\div0.1$=2478.4 U/g

Complex Ⅴ activity of the pellet (U/g)=$128\times y_{Pellet}\div W=128\times1.213\div0.1$=1552.64 U/g

Recommended Products

Catalog No.	Product Name
KTB1851	CheKine™ Plus Micro Mitochondrial Complex Ⅰ Activity Assay Kit
KTB1861	CheKine™ Plus Micro Mitochondrial Complex Ⅱ Activity Assay Kit
KTB1871	CheKine™ Plus Micro Mitochondrial Complex Ⅲ Activity Assay Kit
KTB1881	CheKine™ Plus 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. For your safety and health, please wear a lab coat and disposable gloves.

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