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CheKine™ Micro Acetyl Coenzyme A (Acetyl-CoA) Assay Kit

Cat #: KTB1260 Size: 48 T/48 S 96 T/96 S

	Micro Acetyl Coenzyme A (Acetyl-CoA) Assay Kit		
REF	**Cat #:** KTB1260	LOT	**Lot #:** Refer to product label
	Detection Range: 1.5-3,200 nmol/mL		**Sensitivity:** 1.5 nmol/mL
	Applicable samples: Animal and Plant Tissues, Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Acetyl Coenzyme A (Acetyl-CoA) is widely presented in animals, plants, microorganisms and cultured cells. It is an important intermediate metabolite produced during the metabolism of biometrics. It is a pivotal substance in the metabolism of energy substances in the body. The three major nutrients-glucose, lipid, protein which could be via acetyl coenzyme A to form a common metabolic pathway - tricarboxylic acid circulation and phosphorylation. Through this path, Acetyl-CoA thoroughly could be oxidize carbon dioxide and water, and release energy for ATP synthesis. Furtherly, Acetyl-CoA a is a precursor material for the synthesis of fatty acid, ketone body, and Cholesterol and its derivatives. CheKine™ Micro Acetyl Coenzyme A (Acetyl-CoA) Assay Kit provides a simple, sensitive, rapid colorimetric Acetyl-CoA detection method. Suitable for various types of samples, especially animals or plant tissues or cells. The detection principle is that malate dehydrogenase can catalyze malate acid and NAD to produce oxaloacetic acid and NADH. Citrate synthase can catalyze Acetyl-CoA and oxaloacetic acid to produce citric acid and CoA. Based on the coupling reaction of malate dehydrogenase and citrate synthase, Acetyl-CoA content is proportional to the generating rate of NADH. NADH has a special absorption peak at 340 nm, and the calculation of the absorbance value of 340 nm can be obtained the content of Acetyl-CoA.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	Powder×2 vials	Powder×2 vials	-20℃, protected from light
Reagent Ⅱ	3.5 μL×2	7 μL×2	4℃, protected from light
Reagent Ⅲ	Powder×2 vials	Powder×2 vials	-20℃, protected from light
Reagent Ⅳ	40 mL	40 mL×2	4℃

Standard (NADH)	Powder×2 vials	Powder×2 vials	-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 ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Ice maker, refrigerated centrifuge
- Water bath
- 96-well UV plate or microquartz cuvette
- Precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Extraction Buffer and has a pungent odor, so it is recommended to experiment in a fume hood.

Working Reagent Ⅰ: Reconstitute one vial of Reagent Ⅰ with 175 μL (for 48 T) or 350 μL (for 96 T) of ReagentⅣ. Mix well until fully dissolved and store for subsequent use. Protect from light during the experiment. The remaining Working Reagent Ⅰ can be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing. Single tube of the 48 T can accommodate 48 reactions,single tube of the 96 T can accommodate 96 reactions. A total of two tubes are provided within the kit.

Working Reagent Ⅱ: Reconstitute one vial of Reagent Ⅱ with 87.5 μL (for 48 T) or 175 μL (for 96 T) of ReagentⅣ. Mix well until fully dissolved and store for subsequent use. Protect from light during the experiment. The remaining Working Reagent Ⅱ can be stored at 4℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing. Single tube of the 48 T can accommodate 24 reactions,single tube of the 96 T can accommodate 48 reactions. A total of two tubes are provided within the kit.

Working Reagent Ⅲ: Reconstitute one vial of Reagent Ⅲ with 15.75 mL (for 48 T) or 31.5 mL (for 96 T) of ReagentⅣ. Mix well until fully dissolved and store for subsequent use. Protect from light during the experiment. The remaining Working Reagent Ⅲ can be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing. Single tube of the 48 T can accommodate 48 reactions,single tube of the 96 T can accommodate 96 reactions. A total of two tubes are provided within the kit.

Working Solution: Prepare before use, Mix Working Reagent Ⅰ, Working Reagent Ⅱ and Working Reagent Ⅲ according to 1:1:90 ratio. Working Reagent is freshly prepared.

Standard (NADH): Reconstitute one vial of Standard with 0.5 mL (for 48 T) or 1 mL (for 96 T) of deionized water. Mix well until fully dissolved and store for subsequent use. 8,000 nmol/mL; protect from light during the experiment. The remaining Working Reagent Ⅱ can be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing. Single tube of the 48 T can accommodate 48 reactions,single tube of the 96 T can accommodate 96 reactions. A total of two tubes are provided within the kit.

Standard curve setting: Dilute 8,000 nmol/mL Standard with deionized water to 3,200, 1,600, 800, 400, 200, 100, 50 ,0 nmol/mL standard solution as shown in the table below.

Num.	Standard Volume	Deionized Water（μL）	Concentration（nmol/mL）
Std.1	100 μL 8,000 nmol/mL	150	3,200
Std.2	100 μL of Std.1 (3,200 nmol/mL)	100	1,600
Std.3	100 μL of Std.2 (1,600 nmol/mL)	100	800
Std.4	100 μL of Std.3 (800 nmol/mL)	100	400

Std.5	100 μL of Std.4 (400 nmol/mL)	100	200
Std.6	100 μL of Std.5 (200 nmol/mL)	100	100
Std.7	100 μL of Std.6 (100 nmol/mL)	100	50
Std.8	0	200	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h. Std.8 is Blank Well.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Cells: Collect 5×10^6 cells pellet, and add 1mL Extraction Buffer, Ultrasonic broken cells (power 20%, ultrasonic 3 s, interval 10 s, repeat 30 times), then centrifuge with 13,000 g for 10 min at 4°C, collect the supernatant, stand by on ice, waiting for test.
2. Tissues: Weigh 0.1 g tissue and mix with 1 mL Extraction Buffer. Homogenize on ice. Then centrifuge the homogenate with 13,000 g for 10 min at 4°C. Stand by on ice, waiting for test.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Ultraviolet spectrophotometer was returned to zero with deionized water.
2. Incubate Working Solution for 10 min at 37°C (mammal) or 25°C (other species).
3. Operating table: in the 96-well UV plate or microquartz cuvette, follow the table below to set the test

Reagent	Test Well（μL）	Standard Well（μL）
Sample	25	0
Different Concentration Std.	0	25
Working Solution	230	230

4. Mix well, immediately read 340 nm absorbance value of the test well at 20 s and 1 min and 20 s, recorded as A_1, A_2, respectively. Then calculate the test well $\Delta A_{Test} = A_2 - A_1$. Read 340 nm absorbance value of the standard well at 1 min and 20 s, recorded as $A_{Standard}$, then calculate the standard well, $\Delta A_{Standard} = A_{Standard} - A_{Blank}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. Please reduce the sample quantity appropriately if the OD values is higher than 1.5, or reduce the volume of Extraction Buffer if the OD values is too low.

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.

Take the $\Delta A_{Standard}$ as the x axis and the standard concentration as the y axis, make the standard curve, get the equation, finally, get the y value by calculate the ΔA_{Test} in the equation.

1. Calculated by protein concentration:

Acetyl-CoA (nmol/mg prot)$=(y\times V_{Sample})\div(V_{Sample}\times Cpr)$**$=y\div Cpr$**

2. Calculated by fresh weight of samples:

Acetyl-CoA (nmol/g fresh weight)$=(y\times V_{Sample})\div(W\times V_{Sample}\div V_{Extraction})$**$=y\div W$**

3. Calculated by cell density:

Acetyl-CoA (U/10^4 cells) $=(y\times V_{Sample})\div(500\times V_{Sample}\div V_{Extraction})$**$=y\div 500$**

Where: V_{Sample}: sample volume added, 0.025 mL; Cpr: sample protein concentration, mg/mL; W: sample weight, g; $V_{Extraction}$:

sample extract volume, 1 mL; 500: total number of cells, 5×10^6.

Typical Data

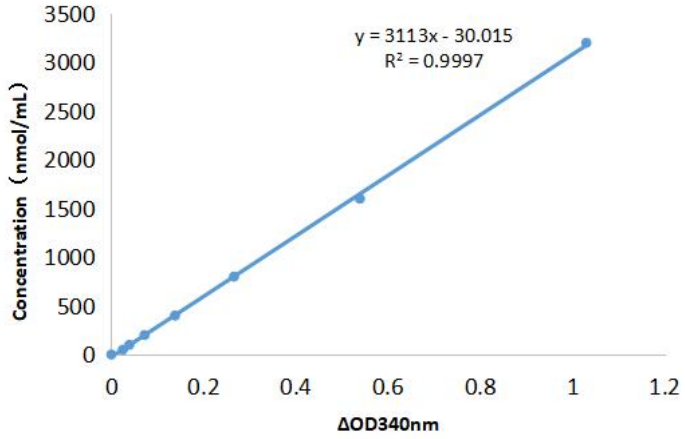

Figure 1. Standard curve of NADH

Recommended Products

Catalog No.	Product Name
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Assay Kit
KTB1250	CheKine™ Micro Mitochondrial Isocitrate Dehydrogenase (ICDHm) 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.