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CheKine™ Micro Aconitase (ACO) Activity Assay Kit

Cat #: KTB1290 Size: 48 T/48 S 96 T/96 S

	Micro Aconitase (ACO) Activity Assay Kit		
REF	**Cat #:** KTB1290	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Aconitase (ACO), an enzyme in the tricarboxylic acid cycle, catalyzes the conversion of citric acid to isocitrate. Citric acid itself is not easy to be oxidized. Under the action of aconitase, the hydroxyl group is transferred from the β carbon atom to the α carbon atom through the reaction of dehydration and water addition to generate isocitric acid that is easy to deoxidize, for further oxidative decarboxylation reaction be prepared. CheKine™ Micro Aconitase (ACO) Activity Assay Kit provides a simple, convenient and rapid ACO activity detection method, which is suitable for the detection of animal tissues, plant tissues, cells, serum, plasma and other samples. The principle is that ACO catalyzes the conversion of citric acid into isocitrate, and the oxidative decarboxylation of isocitrate reduces NAD^+ to NADH, resulting in an increase in light absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Ⅲ	10 mL	20 mL	4℃
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
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 ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

- Ice maker, refrigerated centrifuge, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

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 -20℃, protected from light.

Note: Reagent Ⅱ is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

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

Reagent Ⅳ: Prepare before use. For 48 T, add 2.5 mL deionized water, and for 96 T, add 5 mL deionized water, mix well. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing for one week.

Reagent Ⅴ: Prepare before use. For 48 T, add 0.75 mL of deionized water, and for 96 T, add 1.5 mL of deionized water, mix well. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing for one week.

Working ReagentⅥ: Prepare before use. For 48 T, add 7.2 mL ReagentⅢ to ReagentⅥ, and for 96 T, add 14.4 mL ReagentⅢ to ReagentⅥ, mix well. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing for one week.

Working Solution: The solution was freshly prepared just before use. For 48 T, add 0.6 mL deionized water, 0.6 mL ReagentⅢ, 0.6 mL ReagentⅣ, and 0.6 mL ReagentⅤ to 7.2 mL Working ReagentⅥ; for 96 T, add 1.2 mL deionized water, 1.2 mL ReagentⅢ, 1.2 mL ReagentⅣ, and 1.2 mL ReagentⅤ to 14.4 mL Working ReagentⅥ, and mix well for use. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing for one week.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Plasma and Serum: Direct detection.

2. Extraction of cytoplasmic protein and mitochondrial protein from cells and tissue:

(1) Weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Extraction Buffer and 10 μL ReagentⅡ, homogenize on ice. Centrifuge at 600 g for 5 min at 4℃. Collect the supernatant to a new centrifuge tube and discard the pellet.

(2) Centrifuge the supernatant again at 11,000 g for 10 min at 4℃, and obtain the supernatant and precipitate respectively.

(3) (Optional) The supernatant collected in step 2 is cytoplasmic extract, which can be used to directly determine ACO leaking from mitochondria.

(4) Add 200 μL ReagentⅠ and 2 μL ReagentⅡ to the precipitate collected in step 2, resuspend the precipitate sufficiently, and use it to detect the activity of ACO in the next step.

Note: 1. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the samples are placed for a long time, different operation habits or other reasons, which resulted in a large amount of mMDH in the supernatant, then the supernatant must be tested.

2. The Extraction Buffer of the kit is the same as that of KTB1270, KTB1280, KTB1240, KTB1230, KTB1250, and KTB1023. The samples extracted using this kit are also suitable for analysis with KTB1270, KTB1280, KTB1240, KTB1230, KTB1250, and KTB1023.

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. Add 40 μL of sample, 160 μL of Working Solution in a 96-well UV plate or microquartz cuvette. After mixing quickly, record the absorbance values of 20 s and 3 min 20 s at 340 nm with a microplate reader, mark as A_1 and A_2, and calculate $\Delta A=A_2-A_1$.

Note: If the sample absorbance value ΔA is greater than 0.5, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

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.

A. 96-well UV plates calculation formula

1. Calculation of ACO activity in serum (plasma)

Unit definition: one enzyme activity unit defines as 1 nmol NADH produced by each mL of serum (plasma) per min in the reaction system.

ACO activity (U/mL)=$[\Delta A\times V_{total}\div(\varepsilon\times d)\times10^9]\div V_{sample}\div T$**$=535.90\times\Delta A$**

2. Calculated by fresh weight of samples

Unit definition: one enzyme activity unit defines as 1 nmol NADH produced by 1 g tissue per min in the reaction system.

$ACO_{Supernatant}$ activity (U/g fresh weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Extraction}\times W)\div T$**$=541.26\times\Delta A_{Supernatant}\div W$**

ACO_{Pellet} activity (U/g fresh weight)=$[\Delta A_{Pellet}\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Total\ Sample}\times W)\div T$**$=108.25\times\Delta A_{Pellet}\div W$**

Total ACO activity (U/g fresh weight)=$ACO_{Supernatant}$ activity+ACO_{Pellet} activity**$=541.26\times\Delta A_{Supernatant}\div W+108.25\times\Delta A_{Pellet}\div W$**

3. Calculated by cell density

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

ACO activity (U/10^4 cells)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Total\ Sample}\times500)\div T$**$=0.216\times\Delta A$**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^3 mol/L/cm; d: 0.5 cm; V_{Sample}: sample volume added, 0.04 mL; T: reaction time, 3 min; $\Delta A_{Supernatant}$: OD value of supernatant; $V_{Extraction}$: sample extract volume, 1.01 mL; W: sample weight, g; ΔA_{Pellet}: OD value of pellet; $V_{Total\ Sample}$: the volume of adding Reagents Ⅰ and Ⅱ, 0.202 mL; 500: total number of cells, 5×10^6.

B. Microquartz cuvette calculation formula

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

Recommended Products

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