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CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Activity Assay Kit

Cat #: KTB1240 Size: 48 T/48 S 96 T/96 S

	Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Activity Assay Kit		
REF	**Cat #:** KTB1240	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

α-Ketoglutarate Dehydrogenase (α-KGDH) is widely present in the mitochondria of animals, plants, microorganisms and in-vitro cultured cells. It is one of the key regulation enzymes of Kreb's Cycle, catalyzing the oxidation and decarboxylation of α-Ketoglutarate to produce succinyl coenzyme A. CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Assay Kit provides a simple, sensitive and rapid α-KGDH activity detection method, which is compatible to various biological samples, especially animals or plant tissues, cells. The detection principle is based on that α-KGDH can catalyze α-Ketoglutarate, NAD+ and coenzyme A to produce succinyl-coenzyme A, carbon dioxide and NADH. NADH has a characteristic absorption peak at 340 nm. α-KGDH activity can be calculated according to the generation rate of NADH.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Ⅱ	10.8 mL	21.6 mL	4℃
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅳ	15 μL	30 μL	4℃, protected from light
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	-20℃
Reagent Ⅵ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅶ	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
- Refrigerated centrifuge, water bath, ice maker, 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. Store at -20℃, protected from light.

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

Working Solution: Prepare before use, resolve Reagent Ⅲ, Reagent Ⅳ, Reagent Ⅴ and Reagent Ⅵ all in Reagent Ⅱ, mix well for further use. Working Solution is freshly prepared.

Note: Reagent Ⅳ has certain irritation, so personal protection is recommended during use.

Working Reagent Ⅶ: Prepare before use, resolve Reagent Ⅶ with 0.6 mL deionized water while using 48 T; Resolve Reagent Ⅶ with 1.2 mL deionized water while using 96 T; The remaining reagents should be stored at -20℃, and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

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

1. Animal and Plant Tissues: Weigh approximately 0.1 g of tissue, add 1 mL of Extraction Buffer and 10 μL of Reagent Ⅰ, and homogenize on ice, then centrifuge at 11,000 g for 10 min at 4℃. Collect the supernatant and place it on ice for immediate use or further analysis.
2. Cells: Collect 5 million cells into a centrifuge tube. Wash the cells with cold PBS, then discard the supernatant after centrifugation. Add 1 mL of Extraction Buffer and 10 μL of Reagent Ⅰ. Sonicate the cell suspension on ice for 5 min (power output: 20% or 200 W, 3 s pulse on, 7 s interval, repeated for 30 cycles). After sonication, centrifuge at 11,000 g for 10 min at 4℃. Collect the supernatant and keep it on ice until ready for measurement.

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 10 μL of sample, 10 μL of Working Reagent Ⅶ, and then 180 μL of Working Solution in a 96-well UV plate or microquartz cuvette. After mixing quickly, record the absorbance values of 20 s and 2 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: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately

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. Calculated by fresh weight of samples

Definition of Unit: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of NADH per min per gram of tissue under reaction conditions at 37℃ (for mammals) or 25℃ (for other species).

α-KGDH (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W÷$V_{Total\ Sample}$×V_{Sample})÷T×F**=3,247.59×ΔA÷W×F**

2. Calculated by cell density

Definition of Unit: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of NADH per minute per 10,000 cells under reaction conditions at 37℃ (for mammals) or 25℃ (for other species).

α-KGDH activity (U/10^4 cells)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(N÷$V_{Total\ Sample}$×V_{Sample})÷T×F**=3,247.59×ΔA÷N×F**

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, 0.01 mL; $V_{Total\ Sample}$: Volume of extraction solution added, 1.01 mL; T: Reaction time, 2 min; W: Sample weight, g; N: Total number of cells, 10^4.

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.

Precautions

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.

Recommended Products

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