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

Cat #: KTB1240 Size: 48 T/96 T

	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	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Ⅲ	9 mL	18 mL	4℃
Reagent Ⅳ	1	1	4℃
Reagent Ⅴ	12.5 μL	25 μL	4℃
Reagent Ⅵ	1	1	-20℃
Reagent Ⅶ	1	1	-20℃
Reagent Ⅷ	1	1	-20℃, protected from light

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. 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.

Working Solution: Prepare before use, resolve ReagentⅣ, ReagentⅤ, ReagentⅥ and ReagentⅦ all in ReagentⅢ solution, mix well for further use, store unused Working Solution at 4℃.

Working ReagentⅧ: Prepare before use, resolve ReagentⅧ with 0.5 mL deionized water while using 48 T; Resolve ReagentⅧ with 1 mL deionized water while using 96 T; The remaining reagents should be stored at -20℃, and protected from light 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.

Extraction of cytoplasmic protein and mitochondrial protein from cells, and tissues:

1. Weigh 0.1 g tissues or collect 5×10^6 cells, add 1 mL Extraction Buffer and 10 μL Reagent Ⅱ, 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 determine α-KGDH leaking from mitochondria.
4. Add 200 μL Reagent Ⅰ and 2 μL Reagent Ⅱ to the precipitate collected in step 2, resuspend the precipitate sufficiently, and use it to detect the activity of α-KGDH in the next step.

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, 180 μL of Working Solution, and then 10 μL of Working ReagentⅧ 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

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

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

α-KGDH$_{Pellet}$ activity (U/g fresh weight)=[ΔA_{Pellet}×V_{Total} ÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T**=649.52×ΔA_{Pellet}÷W**

Total α-KGDH activity (U/g fresh weight)=α-KGDH$_{Supernatant}$ activity+α-KGDH$_{Pellet}$ activity**=3,247.59×$\Delta A_{Supernatant}$÷W+649.52×ΔA_{Pellet}÷W**

2. Calculated by cell density

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

α-KGDH activity (U/10^4 cells)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T**=1.3×ΔA**

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; T: reaction time, 2 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 Reagent 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.

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.