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CheKine™ Micro Isocitrate Dehydrogenase Cytoplasmic (ICDHc) Activity Assay Kit

Cat #: KTB1251 Size: 48 T/96 T

	Micro Isocitrate Dehydrogenase Cytoplasmic (ICDHc) Activity Assay Kit		
REF	**Cat #:** KTB1251	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Cytoplasmic isocitrate dehydrogenase (ICDHc, EC 1.1.1.42) is widely distributed in animals, plants, microorganisms, and cultured cells. It catalyzes the decarboxylation and dehydrogenation of isocitrate to produce α-ketoglutarate, while simultaneously reducing $NADP^+$ to NADPH. ICDHc serves as another significant source of NADPH in the cytoplasm, apart from the pentose phosphate pathway, and its activity often undergoes notable changes under stress conditions. CheKine™ Micro Isocitrate Dehydrogenase Cytoplasmic (ICDHc) Activity Assay Kit is capable of detecting cytosolic isocitrate dehydrogenase (ICDHc) activity in animal and plant tissues, cells, or bacteria, as well as in serum (plasma). The principle behind this involves utilizing ICDHc's catalytic action in reducing $NADP^+$ to NADPH, with the subsequent increase in NADPH concentration being measured at a wavelength of 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	9 mL	18 mL	4℃, protected from light
Reagent Ⅱ	1	1	4℃
Reagent Ⅲ	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
- Water bath, ice maker, centrifuge
- 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℃.

Working Solution: Prepare before use, Transfer Reagent Ⅱ to Reagent Ⅰ and mix thoroughly until completely dissolved. The prepared Working Solution can be stored at 4℃ for up to one month.

Working Reagent Ⅲ: Prepare before use, resolve Reagent Ⅲ with 0.5 mL deionized water while using 48 T kit; Resolve Reagent Ⅲ with 1 mL deionized water while using 96 T kit; Unused reagents can be stored at -20℃ in a light-proof condition for 2 weeks, with the caution to avoid repeated freezing and thawing.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Take the supernatant and place it on the ice for testing.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma or serum: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

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 sample, 180 μL Working Solution, and then 10 μL Working Reagent III 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 is less than 0.01, it is advisable to increase the sample volume appropriately or extend the reaction time to 5 min or 10 min. If ΔA is greater than 0.5, 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 as below

1. Calculation of ICDHc activity in serum (plasma)

Active unit definition: 1 nmol NADH generated per min in 1 mL serum (lasma) is defined as a unit of enzyme activity.

$ICDHc\ (U/mL)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div V_{Sample}\div T$**$=3,216\times\Delta A$**

2. Calculation of XXX activity in tissues, bacteria or cells

(1) Calculated by protein concentration

Active unit definition: 1 nmol NADH generated per min in 1mg tissue protein is defined as a unit of enzyme activity.

ICDHc (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(Cpr×V_{Sample})÷T**=3,216×ΔA÷Cpr**

(2) Calculated by sample fresh weight

Active unit definition: 1 nmol NADH generated per min in 1 g tissue is defined as a unit of enzyme activity.

ICDHc (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T**=3,216×ΔA÷W**

(3) Calculated by bacteria or cell number

Active unit definition: 1 nmol NADH generated per min in 10^4 bacteria or cells is defined as a unit of enzyme activity.

ICDHc (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T=3,216×ΔA÷500**=6.432×ΔA**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficien, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 2 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of bacteria or 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

1. 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.

Typical Data

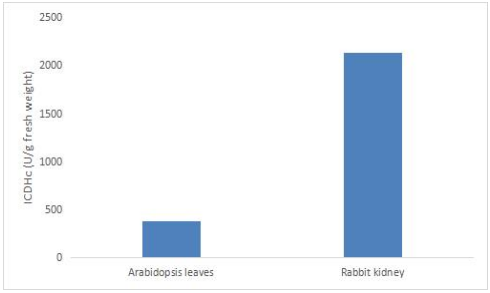

Figure 1. ICDHc activity in Arabidopsis leaves and Rabbit kidney was detected with this kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.