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Cytosolic Isocitrate Dehydrogenase (ICDHc) Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0549
Size:100T/96S

Components:
Extract solution: Liquid 120 mL×1. Storage at 4℃ .
Reagent I: Powder×1. Storage at 4℃ . Dissolve it thoroughly with 20 mL of Extract solution before use.
Reagent II: Powder×1. Storage at 4℃ . Dissolve it thoroughly with 275 μL of distilled water before use.
Reagent III: Powder×1. Storage at 4℃ . Dissolve it thoroughly with 275 μL of distilled water before use.
Working solution: Mix the Reagent I, Reagent II and Reagent III as a ratio of 85:1:1.

Product Description:
ICDHc widely exist in animals, plants, microorganisms and cultured cells, which catalyzes isocitric acid dehydrogenize and decarboxylate to form α-ketoglutaric acid, reduce $NADP^+$ to form NADPH. ICDHc is a source of NADPH except pentose phosphate pathway in cytoplasm, the enzyme activity will change significantly in adversity.
ICDHc catalyzes $NADP^+$ to form NADPH, the activity of ICDHc can be detected by the increase of NADPH concentration at 340 nm.

Reagents and Equipment Required but Not Provided:
Ultraviolet spectrophotometer/microplate reader, constant temperature water bath, micro quartz cuvette/96 well plate (UV plate), desk centrifuge, adjustable pipette, mortar/homogenizer, ice and distilled water.

Sample preparation:
1. Cells or bacteria: Collect bacteria or cells into centrifuge tube, after centrifugation discard supernatant. Suggest 2 million of bacteria or cells with 0.4 mL of Extract solution, splitting with ultrasonic (ice bath, power 20%, work time 3s, interval 10s, for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before test.
2. Tissue: Add 1 mL of Extract solution into 0.1 g of tissue, fully grinding on ice. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before test.
3. Serum (plasma): Detect directly.

Procedure:
1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, set the counter to zero with distilled water.
2. Add the following reagents to micro quartz cuvette/96 well UV plate:

Reagent name (μL)	Test tube (T)

Working solution	190
Sample	10

Add working solution and sample to micro quartz cuvette or 96 well flat-bottom UV plate. Mix thoroughly and timing, measure the absorption at 340 nm at 20s recorded as A1, then put the micro quartz cuvette and react solution to 37℃ water bath for 2 minutes. Take out and dry it quickly, detect the absorbance at 340 nm at 140s, recorded as A2, calculate $\Delta A=\Delta A_2-\Delta A_1$.

Calculation:

A. micro quartz cuvette:

a. Serum (plasma)

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per min in react system every milliliter of serum (plasma).

ICDHc (U/mL)=(ΔA÷d÷ε×Vrv×10^9)÷Vs÷T=1608×ΔA

b. Tissue:

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per min in react system every milligram of tissue protein.

ICDHc (U/mg prot)=(ΔA÷d÷ε×Vrv×10^9)÷(Cpr×Vs)÷T=1608×ΔA ÷Cpr

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per min in react system every gram of tissue.

ICDHc (U/g weight)=(ΔA÷d÷ε×Vrv×10^9)÷(W÷Ve×Vs)÷T=1608×ΔA ÷W

c. Bacteria or cells:

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per min in react system every milligram of tissue protein.

ICDHc (U/mg prot)=(ΔA÷d÷ε×Vrv×10^9)÷(Cpr×Vs)÷T=1608×ΔA ÷Cpr

2)Density of bacteria or cell:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per min in react system every 10000 bacteria or cells.

ICDHc (U/10^4 cell)=(ΔA÷d÷ε×Vrv×10^9)÷(500×Vs)÷T=3.2×ΔA

Cpr: Sample protein concentration (mg/mL);

W: Sample weight(g);

Vs: Enzyme solution volume (mL), 0.01 mL;

Ve: Extract solution added volume(mL), 1 mL;

Vrv: Total reaction volume, 1 mL;

T: Reaction time (min), 2 minutes;

500: Cells or bacteria amount, 5 million;

d: light diameter, 1 cm;

ε: ICDHc extinction coefficient, 6.22×10^3 L/mol/cm.

B. 96 well flat-bottom plate

The light diameter of the 96 well flat-bottom UV plate is 0.6 centimeter, change the light diameter in the formula of micro quartz cuvette from 1 to 0.6.

Note:

1. Dilute enzyme solution with Extract solution if A2-A1>0.5 or A1>0.5 to make it less than 0.5, which can improve detect sensitivity.
2. Put Reagent II and III on the ice to avoid denaturation and inactivation, put working solution in 37℃ water bath.
3. Keep 37℃ of the react solution in cuvette, add 37℃ water to a beaker, put this beaker in 37℃ water bath and put the cuvette in this beaker.
4. It is better for two people to do this experiment at the same time, one for colorimetric and the other for timing to ensure the accuracy of the experimental results.

Experimental Examples：

1. Take 0. 1g of Echinochloa crusgalli, add 1ml extract, homogenize in ice bath, then centrifuge at 8000g and 4℃ for 10 min, take the supernatant, then operate according to the determination steps, measure and calculate ΔA= A2-A1 =0.2285-0.2139 = 0.0146 with micro quartz cuvette, and calculate the enzyme activity according to the sample mass

Icdhc (U/g mass) = 1608 × ΔA ÷ W = 234.768 U/g mass.

2. Take 0. 1g of mouse kidney tissue, add 1ml extract, homogenize it in ice bath, then centrifuge at 8000g and 4℃ for 10min, take the supernatant and dilute it 10 times, then operate according to the determination steps, measure and calculate ΔA= A2-A1 = 0.5989-0. 1263 = 0.4726 with micro quartz plate, and calculate the enzyme activity according to the sample mass

Icdhc (U/g mass) = 1608 × ΔA ÷ W × 10 = 75994.08 U/g mass.

References：

[1] Miake F, TORIKATA T, KOGA K, et al. Isolation and characterization of NADP+-specific isocitrate dehydrogenase from the pupa of Bombyx mori[J]. The Journal of Biochemistry, 1977, 82(2): 449-454.

Related Products：

AK0488/AK0487	NADP Phosphatase(NADPase) Activity Assay Kit
AK0570/AK0569	G6PDH Activity Assay Kit
AK0486/AK0485	NADP Malic Enzyme(NADP-ME) Activity Assay Kit
AK0484/AK0483	NAD Malic Enzyme(NAD-ME) Activity Assay Kit
AK0408/AK0407	6 PGDH Activity Assay Kit