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Cytosolic Isocitrate Dehydrogenase (ICDHc) Assay Kit

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

Cat No: AK0550
Size:50T/48S

Components:
Extract solution: Liquid 100 mL×1. Storage at 4℃ .
Reagent I: Powder×1. Storage at 4℃ . Dissolve it thoroughly with 50 mL of Extract solution before use.
Reagent II: Powder×2. Storage at 4℃ . Dissolve it thoroughly with 275 μL of distilled water before use.
Reagent III: Powder×2. Storage at 4℃ . Dissolve it thoroughly with275 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, 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, constant temperature water bath, desk centrifuge, adjustable pipette, 1mL quartz cuvette, 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 spectrophotometer for 30 minutes, set the counter to zero with distilled water.
2. Add the following reagents to 1mL glass cuvette:

Reagent	Test tube (T)
Working solution (μL)	950

Sample (μL)	50

Add working solution and sample to 1 mL quartz cuvette. Mix thoroughly and timing, measure the absorption at 340 nm at 20s recorded as A1, then put the cuvette and react solution to 37℃ water bath for 2 minutes. Take out and dry it quickly, detect the absorbance at 340 nm at 2min20s, recorded as A2, calculate $\Delta A=\Delta A_2-\Delta A_1$.

Calculation:

1) 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

2) Tissue:

A. 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

B. 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

3) Bacteria or cells:

A. 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

B. 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 concentration (mg/mL);

W: Sample weight(g);

Vs: Enzyme solution volume (mL), 0.05 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/mL;

d: Light path, 1 cm;

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

Note:

1. Dilute enzyme 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 1 mL of 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 =0.240-0.224=0.016 with micro quartz cuvette, and calculate the enzyme activity according to the sample mass

Icdhc (U/g mass) = 1608 × ΔA ÷ W = 257.28 U/g mass.

2. Take 0. 1g of mouse kidney tissue, add 1 mL of 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 = 0.253-0. 141=0. 112 with micro quartz plate, and calculate the enzyme activity according to the sample mass

Icdhc (U/g mass) = 1608 × ΔA ÷ W × 10 = 9004.8 U/g mass.

3. Take the mouse serum samples for direct detection, and calculate ΔA =A2-A1 = 0.225-0.2 = 0.025

ICDHc(U/mL) =1608×ΔA=40.2 U/mL.

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