Cytosolic Isocitrate Dehydrogenase (ICDHc) Activity Assay Kit

Operation Equipment: Spectrophotometer

Catalog Number: BC0400

Size:50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 100 mL×1	2-8℃
Reagent I	Powder×1	2-8℃
Reagent II	Powder×2	2-8℃
Reagent III	Powder×2	2-8℃

Solution Preparation:

1. **Reagent I:** Dissolve it thoroughly with 50 mL of Extract solution before use.
2. **Reagent II:** Dissolve it thoroughly with 275 μL of distilled water before use.
3. **Reagent III:** Dissolve it thoroughly with275 μL of distilled water before use.
4. **Working solution:** Mix the Reagent I, Reagent II and Reagent III as a ratio of 85:1:1, and prepare them as needed.

Product Description:

ICDHc (EC 1.1.1.42) 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, centrifuge, adjustable pipette, 1 mL quartz cuvette, mortar/homogenizer/cell ultrasonic crusher, 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 200W, 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 for 30 minutes, adjust the wavelength to 340 nm, 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 ΔA=A2-A1.

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 protein 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: NADPH 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 crus-galli*, add 1 mL of Extraction solution, homogenize in ice bath, then centrifuge at 8000 ×g 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 1 mL quartz cuvette, and calculate the enzyme activity according to the sample weight:
   ICDHc (U/g weight) = 1608 × ΔA ÷ W = 257.28 U/g weight.
2. Take 0.1g of mouse kidney tissue, add 1 mL of Extraction solution, homogenize it in ice bath, then centrifuge at 8000 ×g and 4℃ for 10 min, take the supernatant and dilute it 5 times, then operate according to the determination steps, measure and calculate ΔA= A2-A1 = 0.253-0.141=0.112 with 1 mL quartz plate, and calculate the enzyme activity according to the sample weight:
   ICDHc (U/g weight) = 1608 × ΔA ÷ W × 5 = 9004.8 U/g weight.
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.

Recent Product Citations:

[1] Jiang Y, Cao S, Zhou B, Cao Q, Xu M, Sun T, Zhao X, Zhou Z, Wang Y. Hemocytes in blue mussel Mytilus edulis adopt different energy supply modes to cope with different BDE-47 exposures. Sci Total Environ. 2023 Aug 10;885:163766. doi: 10.1016/j.scitotenv.2023.163766. Epub 2023 May 3. PMID: 37146804.

[2] Yuan Z, Wang J, Che R, God'spower BO, Zhou Y, Dong C, Li L, Chen M, Eliphaz N, Liu X, Li Y.

Relationship between L-lactate dehydrogenase and multidrug resistance in Staphylococcus xylosus. Arch Microbiol. 2021 Dec 28;204(1):91. doi: 10.1007/s00203-021-02625-8. PMID: 34962581.

[3] Zhou Y, Liu X, Huang C, Lin D. Lactate Activates AMPK Remodeling of the Cellular Metabolic Profile and Promotes the Proliferation and Differentiation of C2C12 Myoblasts. Int J Mol Sci. 2022 Nov 13;23(22):13996. doi: 10.3390/ijms232213996. PMID: 36430479; PMCID: PMC9694550.

[4] Zhan S, Zhang Q, Yao Y, Cui Y, Huang T. Cytosolic isocitrate dehydrogenase regulates plant stem cell maintenance in response to nutrient deficiency. Plant Physiol. 2023 Aug 3;192(4):3069-3087. doi: 10.1093/plphys/ kiad246. PMID: 37086475.

[5] Zhang X, Wei X, Ali MM, Rizwan HM, Li B, Li H, Jia K, Yang X, Ma S, Li S, Chen F. Changes in the Content of Organic Acids and Expression Analysis of Citric Acid Accumulation-Related Genes during Fruit Development of Yellow (Passiflora edulis f. flavicarpa) and Purple (Passiflora edulis f. edulis) Passion Fruits. Int J Mol Sci. 2021 May 28;22(11):5765. doi: 10.3390/ijms22115765. PMID: 34071242; PMCID: PMC8198880.

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.

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[3] Kil IS, Lee YS, Bae YS. et al. Modulation of NADP (+)-dependent isocitrate dehydrogenase in aging [J]. Redox Report: Communications in Free Radical Research, 2004, 9(5): 271-277.

[4] Popova T, Pinheiro MA, Matasova L. et al. Regulation of mitochondrial NADP-isocitrate dehydrogenase in rat heart during ischemia [J]. Molecular and Cellular Biochemistry, 2007, 294(1-2): 97-105.

Related Products:

BC1110/BC1115	NADP Phosphatase(NADPase) Activity Assay Kit
BC0260/BC0265	G6PDH Activity Assay Kit
BC1120/BC1125	NADP Malic Enzyme(NADP-ME) Activity Assay Kit
BC1130/BC1135	NAD Malic Enzyme(NAD-ME) Activity Assay Kit

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