

Cytosolic Isocitrate Dehydrogenase (ICDHc) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC0405

Size: 100T/96S

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 120 mL×1	2-8°C
Reagent I	Powder×1	2-8°C
Reagent II	Powder×1	2-8°C
Reagent III	Powder×1	2-8°C

Solution Preparation:

- Reagent I:** Dissolve it thoroughly with 20 mL of Extract solution before use.
- Reagent II:** Dissolve it thoroughly with 275 μ L of distilled water before use.
- Reagent III:** Dissolve it thoroughly with 275 μ L of distilled water before use.
- 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 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, centrifuge, adjustable pipette, micro quartz cuvette/96 well UV plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Sample preparation:

- 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°C 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°C 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°C water bath for 2 minutes. Take out and dry it quickly, detect the absorbance at 340 nm at 140s, recorded as A2, calculate $\Delta A = A2 - A1$.

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).

$$ICDHc (U/mL) = (\Delta A \div d \div \epsilon \times V_{rv} \times 10^9) \div V_s \div T = 1608 \times \Delta A$$

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.

$$ICDHc (U/mg prot) = (\Delta A \div d \div \epsilon \times V_{rv} \times 10^9) \div (C_{pr} \times V_s) \div T = 1608 \times \Delta A \div C_{pr}$$

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.

$$ICDHc (U/g weight) = (\Delta A \div d \div \epsilon \times V_{rv} \times 10^9) \div (W \div V_e \times V_s) \div T = 1608 \times \Delta A \div W$$

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.

$$\text{ICDHc (U/mg prot)} = (\Delta A \div d \div \varepsilon \times V_{rv} \times 10^9) \div (\text{Cpr} \times V_s) \div T = 1608 \times \Delta A \div \text{Cpr}$$

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.

$$\text{ICDHc (U/10}^4 \text{ cell)} = (\Delta A \div d \div \varepsilon \times V_{rv} \times 10^9) \div (500 \times V_s) \div T = 3.2 \times \Delta A$$

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, 2×10^{-4} L;

T: Reaction time (min), 2 minutes;

500: Cells or bacteria amount, 5 million;

d: light diameter, 1 cm;

ε : NADPH 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 $A_2 - A_1 > 0.5$ or $A_1 > 0.5$ 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°C water bath.
3. Keep 37°C of the react solution in cuvette, add 37°C water to a beaker, put this beaker in 37°C 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 1ml Extraction solution, homogenize in ice bath, then centrifuge at $8000 \times g$ and 4°C for 10 min, take the supernatant, then operate according to the determination steps, measure and calculate $\Delta A = A_2 - A_1 = 0.2285 - 0.2139 = 0.0146$ with micro quartz cuvette, and calculate the enzyme activity according to the sample weight:

$$\text{ICDHc (U/g weight)} = 1608 \times \Delta A \div W = 234.768 \text{ U/g weight.}$$

2. Take 0.1g of mouse kidney tissue, add 1ml extract, homogenize it in ice bath, then centrifuge at $8000 \times g$ and 4°C for 10min, take the supernatant and dilute it 10 times, then operate according

to the determination steps, measure and calculate $\Delta A = A_2 - A_1 = 0.5989 - 0.1263 = 0.4726$ with micro quartz cuvette, and calculate the enzyme activity according to the sample weight:
 $\text{ICDHc (U/g weight)} = 1608 \times \Delta A \div W \times 10 = 75994.08 \text{ U/g weight.}$

Recent Product Citations:

[1] 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.

[2] 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.

[3] Xu R, Xu P, Wei H, Huang Y, Zhu X, Lin C, Yan Z, Xin L, Li L, Lv W, Zeng S, Tian G, Ma J, Cheng B, Lu H, Chen Y. Ticlopidine induces embryonic development toxicity and hepatotoxicity in zebrafish by upregulating the oxidative stress signaling pathway. *Ecotoxicol Environ Saf.* 2023 Jul 31; 262:115283. doi: 10.1016/j.ecoenv.2023.115283. Epub ahead of print. PMID: 37531924.

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.

[2] Yang JH, Yang ES, Park JW. Inactivation of NADP⁺-dependent isocitrate dehydrogenase by lipid peroxidation products [J]. *Free Radical Research*, 2004, 38(3): 241-249.

[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
BC2100/BC2105	6PGDH Activity Assay Kit