Isocitrate Dehydrogenase Mitochondrial(ICDHm) Activity Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: BC2165

Size: 100T/48S

Components:

Extract solution I: Liquid 60mL×1, stored at 2-8℃;

Extract solution II: Liquid 600 μL×2, stored at -20°C and protect from light; volatile reagents, cover tightly after use and return to -20°C in time.

Extract solution III: Liquid 40 mL×1 bottle, stored at 2-8°C.

Reagent I: Liquid 5 mL×1, stored at 2-8°C.

Reagent II: Liquid 5 mL×1, stored at room temperature and protect from light.

Reagent III: powder×2, stored at -20°C and protect from ligh; Add 0.375 mL of distilled water before use, fully dissolve the reagents for later use, and store unused reagents in aliquots at -20°C for 4 weeks, avoiding repeated freezing and thawing.

Reagent IV: Liquid 5 mL×1, stored at room temperature.

Reagent V: Liquid 15 mL × 1, stored at room temperature and protect from light;

Standard: powder×1, stored at 2-8°C and protect from light. 10 mg of α-ketoglutarate. Just before use, 684 μL of distilled water is added to prepare a 100 μmol/mL standard solution. Unused reagents can be stored at 2-8°C for 8 weeks.

Preparation of working solution: Reagent I and Reagent II are mixed at a ratio of 1: 1 according to the amount before use.

Product Description:

Isocitrate dehydrogenase (ICDHm) is widely present in mitochondria of animals, plants, microorganisms and cultured cells, and is related to mitochondrial gene expression and other functions of mitochondria. There are two forms of isocitrate dehydrogenase in the body, NAD-dependent isocitrate dehydrogenase using NAD as a coenzyme, and NADP-dependent isocitrate dehydrogenase using NADP as a coenzyme.

The main function of isocitrate dehydrogenase is which catalyze the production of α-ketoglutarate from isocitrate during the tricarboxylic acid cycle in the body, reduce NAD to NADH. Isocitrate dehydrogenase activity could be calculate by determine the amount of α-ketoglutarate produced.

Required material

Centrifuge, spectrophotometer/plate reader, water bath/incubator, micro glass cuvettes/96-well plate, adjustable pipette, mortar/homogenizer, ice and distilled water.

Procedure:

I. Extraction of Mitochondrial Isocitrate Dehydrogenase

1. Weigh about 0.2 g of tissue or collect 10 million cells, add 1 mL of Extract solution I and 10 μL of

Extract solution II, and homogenize with an ice bath homogenizer or mortar. Centrifuge at 1000 g for 10 min at 4°C.

2. Transfer the supernatant to another centrifuge tube and centrifuge at 11000 g and 4°C for 15 min.
3. The supernatant is the cytoplasmic extract, which can be used to determine the isocitrate dehydrogenase leaking from the mitochondria (this step is optional, you can judge the effect of mitochondrial extraction).
4. Add 400 μL of Extract solution III and 4 μL of Extract solution II to the pellet, sonicate (power 300w, sonicate for 5 seconds, interval of 9 seconds, 4 min), centrifuge at 10000 g and 4 °C for 10 min, and take the supernatant for isocitrate dehydrogenase activity measurement and protein content determination.

Determination procedure:

1 Preheat the spectrophotometer/microplate reader 30 min, adjust wavelength to 505 nm, spectrophotometer set zero with distilled water.

2 Dilute the standard with the **Extract solution III** to 0.6, 0.3, 0.15, 0.075, 0.0375, 0.01875 μmol/mL standard solution.

3 Add reagents with the following list:(Perform the following in a 0.6 mL EP tube/96well flat-bottom plate)

Reagent name (μL)	Control tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Supernatant	40	40	-	
Standard solution			40	
Working solution	40	40	40	40
Reagent III	-	4	4	4
Distilled water	4			40
Mix well and place in a 37°C water bath/37°C incubator for 1 h.				
Reagent IV	20	20	20	20
Mix well and place in a 37°C water bath/37°C incubator for 10 min..				
Reagent V	96	96	96	96
Mix well, let stand at room temperature for 5 minutes, and measure the absorbance at 505 nm as soon as possible, and record them as A_T, A_C, A_S, A_B, and calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.				

Note: The blank tube only needs to be measured once or twice.

II. Calculation:

1. Standard curve drawing:

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y=kx+b, and bring ΔA into the equation to get x (μmol/mL).

2. Calculation of enzyme activity

Unit definition : One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of α-ketoglutarate in the reaction system per minute every mg protein.

ICDHm enzyme activity (U/mg prot) = $x \times V_{SR} \div (Cpr \times V_{SR}) \div T \times 10^3 = x \div Cpr \times 16.67$

V_{SR}: add the volume of supernatant, 0.04 mL;

Cpr: sample protein concentration, mg/mL, need to determine by yourself, our company's BCA protein concentration determination kit is recommended;

V_{TV}: reaction system total volume, 0.2 mL;

T : Reaction time, 1 h = 60 min;

10^3: unit conversion factor, 1 μmol = 10^3 nmol.

Note:

1. To ensure the accuracy of the experimental results, you need to take 1-2 samples for preliminary experiments. If the measured absorbance is too high (higher than 1), you can use the extraction solution to dilute the supernatant before measuring. When calculating the results, pay attention to multiplying by the dilution factor.
2. It is recommended to use the sample protein concentration to calculate the enzyme activity. If you use the fresh weight of the sample, you need to determine the enzyme activity of the cytosolic extract. The sum of the supernatant and the precipitated enzyme activity is the total enzyme activity.
3. When measuring the protein concentration, since the Reagent I itself contains protein (about 1 mg/mL), this part of the protein needs to be deducted during the measurement.
4. Attachment: the formula for calculating the fresh weight of the sample:

A. Calculation of ICDHm activity in supernatant (cytoplasm):

Calculated by sample fresh weight:

Unit definition : One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of α-ketoglutarate in the reaction system per minute every g sample.

ICDHm activity (U/g weight) =$x \times V_S \div (W \times V_S \div V_E) \div T \times 10^3 = 16.83 \times x \div W$

V_E: volume of extraction solution added, 1.01 mL;

V_S: volume of supernatant added, 0.04 mL;

W: fresh weight of sample, g;

T: reaction time, 1 h = 60 min;

10^3: unit conversion factor, 1 μmol = 10^3 nmol.

B. Calculation of ICDHm activity in precipitation (mitochondria):

Calculated by sample fresh weight:

Unit definition : One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of α-ketoglutarate in the reaction system per minute every g sample.

ICDHm activity (U/g weight) = $x \times V_S \div (W \times V_S \div V_E) \div T \times 10^3 = 6.73 \times x \div W$

V_E: add the volume of extraction solution when the pellet is resuspended, 0.404 mL;

V_S: add the volume of supernatant solution, 0.04 mL;

W: fresh sample weight, g;

T: reaction time, 1 h = 60 min;

10^3: unit conversion Coefficient, 1 μmol = 10^3 nmol.

C. Sample ICDHm total vitality calculation:

The total ICDHm activity in the sample is the sum of the ICDHm activity in the supernatant (cytoplasm) and the ICDHm activity in the precipitate (mitochondria).

Calculated by sample fresh weight:

ICDHm (U/g weight) =16.83×x÷W+6.73×x÷W

Experimental example:

1. Take 0.2g mouse kidney, add 1.5 mL Extract solution I and 15 μL of Extract solution II, and homogenize with ice bath homogenizer. Centrifugation at 4℃ for 10 min. The supernatant is transferred to another centrifuge tube and centrifuged at 4℃ and 11000g for 15 min. The supernatant is the cytoplasmic extract. 600 μL of Extract solution III and 6 μL of Extract solution II are added to the precipitation. The supernatant is broken by ultrasonic wave and centrifuged at 4℃ for 10 min. the supernatant is detected according to the operation steps. The results are as follows: the determination of cytoplasmic ΔA =A_T-A_C = 0.25-0.25 = 0, the determination of linear ΔA=A_T-A_C = 0.433-0.279 = 0.154, and the standard curve y = 0.5533x + 0.0319.

   The ICDHm activity in cytoplasm (U/g mass) = 16.83 × x ÷ W = 0 U/g mass
   the ICDHm activity in mitochondria (U/g mass) = 6.73 × x ÷ W = 7.43 U/g mass
   the total ICDHm (U/g mass) = 16.83 × x ÷ W + 6.73 × x ÷ W = 7.43 U/g mass.

2. Take 0.3g of ryegrass, add 1.5 mL of Extract solution I and 15 μL of Extract solution II, and homogenize with ice bath homogenizer. Centrifugation at 4℃ for 10 min. The supernatant is transferred to another centrifuge tube and centrifuged at 4℃ and 11000g for 15 min. The supernatant is the cytoplasmic extract. The supernatant is diluted 2 times. 600 μL of Extract solution III and 6 μL of Extract solution II are added to the precipitate. The supernatant is broken by ultrasonic wave and centrifuged at 4℃ for 10 min. The supernatant is diluted 2 times and detected by 96 well plate. The results showed that: cytoplasmic ΔA_T= A_T-A_C = 0.377-0.34 = 0.037, mitochondrial ΔA_T = A_T-A_C = 0.711-0.649 = 0.062, The standard curve y = 0.5533x + 0.0319 is used to calculate the x value, and the enzyme activity is calculated according to the sample mass:

   ICDHm activity in cytoplasm (U/g mass) = 16.83×x÷W×2 = 1.55 U/g mass,
   ICDHm activity in mitochondria (U/g mass) = 6.73× x ÷W×2 = 3.66 U/g mass,
   total ICDHm (U/g mass) = 16.83×x ÷W×2 + 9.16×x ÷W×2 = 5.21 U/g mass.

Recent product Citations：

[1] Xiao Li,Qi Zhao,Jianni Qi,et al. lncRNA Ftx promotes aerobic glycolysis and tumor progression through the PPARγ pathway in hepatocellular carcinoma. International Journal of Oncology. May 2018;(IF3.571)

References：

[2] Igamberdiev A U, Gardeström P. Regulation of NAD-and NADP-dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves[J]. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2003, 1606(1-3): 117-125.

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