Isocitrate Dehydrogenase Mitochondrial(ICDHm) Activity Assay Kit

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

Detection instrument: Spectrophotometer

Cat No: BC2160

Size: 50T/24S

Components:

Extract solution I: Liquid 45 mL×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℃ in time.

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

Reagent I: Liquid 10 mL×1, stored at 2-8°C.

Reagent II: Liquid 10 mL×1, stored at room temperature and protect from light.

Reagent III: powder×2, stored at -20°C and protect from ligh; Add 0.75 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 10 mL×1, stored at room temperature.

Reagent V: Liquid 35 mL × 1, stored at room temperature;

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, water bath/incubator, 1mL glass cuvettes, adjustable pipette, mortar/homogenizer, ice, and distilled water.

Procedure:

I. Extraction of Mitochondrial Isocitrate Dehydrogenase

1. Weigh about 0.3 g of tissue or collect 15 million cells, add 1.5 mL of Extract solution I and 15 μL of

Extract solution II, and homogenize with an ice bath homogenizer or mortar.

2. Centrifuge at 1000 g for 10 min at 4°C.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 600 μL of Extract solution III and 6 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 mitochondrial isocitrate dehydrogenase activity measurement and protein content determination.

II. Determination procedure:

1 Preheat the spectrophotometer 30 min, adjust wavelength to 505 nm, 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 1.5 mL EP tube)

Reagent name（μL）	Control tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Supernatant	200	200	-	
Standard solution			200	
Working solution	200	200	200	200
Reagent III	-	20	20	20
Distilled water	20			200
Mix well and place in a 37°C water bath/37°C incubator for one hour.				
Reagent IV	100	100	100	100
Mix well and place in a 37°C water bath/37°C incubator for 10 min..				
Reagent V	480	480	480	480
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.

III.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, 0.2 mL;

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

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 the sample mass is used to calculate the enzyme activity, the enzyme activity of the cytoplasmic extract needs to be added. The sum of the enzyme activity of the supernatant and the precipitate is the total enzyme activity.
3. When measuring the protein concentration, since the reagent 1 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) =$x \times V_S \div (W \times V_S \div V_E) \div T \times 10^3 = 25.25 \times x \div W$

V_E: volume of extraction solution added, 1.515 mL;

V_S: volume of supernatant added, 0.2 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) = $x \times V_S \div (W \times V_S \div V_E) \div T \times 10^3 = 10.1 \times x \div W$

V_E: add the volume of extraction solution when the pellet is resuspended, 0.606 mL;

V_S: add the volume of supernatant solution, 0.2 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) = 25.25×x÷W+10.1×x÷W

Experimental example:

1. Take 0.3g 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 $\Delta A = A_T - A_C = 0$, the determination of linear $\Delta A = A_T - A_C = 0.767 - 0.475 = 0.292$, and the standard curve y = 1.0917x + 0.0471.

   The ICDHm activity in cytoplasm (U/g mass) = 25.25 × x ÷ W = 0 U/g mass,
   the ICDHm activity in mitochondria (U/g mass) = 10.1 × x ÷ W =7.55 U/g mass
   the total ICDHm (U/g mass) = 25.25 × x ÷ W + 10.1 × x ÷ W =7.55U/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. The results showed that: cytoplasmic $\Delta A_T = A_T - A_C = 0$, mitochondrial $\Delta A_T = A_T - A_C = 0.635 - 0.487 = 0.148$, The standard curve y =1.0917x + 0.0471 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) = 25.25×x÷W×2 = 15.49 U/g mass,
   ICDHm activity in mitochondria (U/g mass) = 10.1× x ÷W×2 = 2.28 U/g mass
   total ICDHm (U/g mass) = 25.25×x ÷W×2 + 10.1×x ÷W×2 = 15.49 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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