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 1: 45 mL×1, stored at 4°C;
Extract solution 2: 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 3: 40 mL×1 bottle, stored at 4°C;
Reagent 1: powder×1, stored at 4°C; 10 mL of Reagent 3 is added just before use, and fully dissolved before use;
Reagent 2: powder×1, stored at room temperature and protect from light; add 10 mL of Reagent 3 immediately before use, and fully dissolve until use;
Reagent 3: 20 mL×1, stored at 4°C;
Reagent 4: powder×2, stored at -20°C and protect from light; add 0.75 mL of distilled water just before use, fully dissolve until use;
Reagent 5: 10 mL × 1, stored at room temperature and protect from light;
Reagent 6: 35 mL × 1, stored at room temperature;
Preparation of working solution: Reagent 1 and Reagent 2 are mixed at a ratio of 1: 1 according to the amount before use.
Standard: powder×1, stored at 4°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.

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

Low temperture centrifuge, spectrophotometer, water bath/constant temperature incubator, mortar/ homogenizer, 1 mL glass cuvette, transferpettor, 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 1 and 15 μL of Extract solution 2, and homogenize with an ice bath homogenizer or mortar.

2. Centrifuge at 1000 g for 10 min at 4°C.

3. Transfer the supernatant to another centrifuge tube and centrifuge at 11000 g and 4°C for 15 min.

4. 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).

5. Add 600 μL of Extract solution 3 and 6 μL of Extract solution 2 to the pellet, sonicate (power 40%, 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 3 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 4	-	20	20	20
Distilled water	20			200
Mix well and place in a 37°C water bath/37°C incubator for one hour.				
Reagent 5	100	100	100	100

Mix well and place in a 37°C water bath/37°C incubator for 10 min..				
Reagent 6	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 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. 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) = $25.25\times x\div W+10.1\times x\div W$