

## 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:** AK0248

**Size:** 100T/48S

### Components:

Extract solution 1: 60 mL×1, stored at 4°C;

Extract solution 2: 0.6 mL×2, stored at -20°C and protect from light; avoid repeated freeze-thaw cycles;

Extract solution 3: 40 mL×1 bottle, stored at 4°C;

Reagent 1: Powder×1, stored at 4°C; 5 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 5 mL of Reagent 3 immediately before use, and fully dissolve until use;

Reagent 3: 10 mL×1, stored at 4°C;

Reagent 4: Powder×2, stored at -20°C and protect from light; add 0.375 mL of double distilled water just before use, fully dissolve until use;

Reagent 5: 5 mL × 1, stored at room temperature and protect from light;

Reagent 6: 15 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  $\alpha$ -ketoglutarate. Just before use, 684  $\mu$ L of distilled water is added to prepare a 100  $\mu$ mol/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  $\alpha$ -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  $\alpha$ -ketoglutarate produced.

### Required material

Low temperture centrifuge, spectrophotometer/microplate reader, water bath/constant temperature incubator, mortar/homogenizer, micro glass cuvette/96 well flat-bottom plate, transferpettor, 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 1 and 10  $\mu$ L of Extract solution 2, 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  $\mu$ L of Extract solution 3 and 4  $\mu$ L 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 isocitrate dehydrogenase activity measurement and protein content determination.

### Determination procedure:

- 1 Preheat the spectrophotometer/microplate reader 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  $\mu$ mol/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 ( $\mu$ L)	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 4	-	4	4	4
Distilled water	4			40
Mix well and place in a 37°C water bath/37°C incubator for 1 h.				
Reagent 5	20	20	20	20
Mix well and place in a 37°C water bath/37°C incubator for 10 min..				
Reagent 6	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  $\Delta A_S$  as the y-axis, draw a standard curve to get the standard equation  $y=kx+b$ , and bring  $\Delta A$  into the equation to get x ( $\mu$ mol/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  $\alpha$ -ketoglutarate in the reaction system per minute every mg protein.

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

$V_{SR}$  : 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  $\mu\text{mol}$  =  $10^3$  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  $\alpha$ -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  $\mu\text{mol}$  =  $10^3$  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  $\alpha$ -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  $\mu\text{mol}$  =  $10^3$  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:

$$\text{ICDHm (U/g weight)} = 16.83 \times x \div W + 6.73 \times x \div W$$

#### Experimental example:

1. Take 0.2g mouse kidney, add 1.5 mL Extract solution 1 and 15  $\mu\text{L}$  of Extract solution 2, and homogenize with ice bath homogenizer. Centrifugation at  $4^{\circ}\text{C}$  for 10 min. The supernatant is transferred to another centrifuge tube and centrifuged at  $4^{\circ}\text{C}$  and 11000g for 15 min. The supernatant is the cytoplasmic extract. 600  $\mu\text{L}$  of Extract solution 3 and 6  $\mu\text{L}$  of Extract solution 2 are added to the precipitation. The supernatant is broken by ultrasonic wave and centrifuged at  $4^{\circ}\text{C}$  for 10 min. the supernatant is detected according to the operation steps. The results are as follows: the determination of cytoplasmic  $\Delta A = A_T - A_C = 0.25 - 0.25 = 0$ , the determination of linear  $\Delta A = A_T - A_C = 0.433 - 0.279 = 0.154$ , and the standard curve  $y = 0.5533x + 0.0319$ . The ICDHm activity in cytoplasm (U/g mass) =  $16.83 \times x \div W = 0$  U/g mass, the ICDHm activity in mitochondria (U/g mass) =  $6.73 \times x \div W = 7.43$  U/g mass, and the total ICDHm (U/g mass) =  $16.83 \times x \div W + 6.73 \times x \div W = 7.43$  U/g mass.

2. Take 0.3g of ryegrass, add 1.5 mL of Extract solution 1 and 15  $\mu\text{L}$  of Extract solution 2, and homogenize with ice bath homogenizer. Centrifugation at  $4^{\circ}\text{C}$  for 10 min. The supernatant is transferred to another centrifuge tube and centrifuged at  $4^{\circ}\text{C}$  and 11000g for 15 min. The supernatant is the cytoplasmic extract. The supernatant is diluted 2 times. 600  $\mu\text{L}$  of Extract solution 3 and 6  $\mu\text{L}$  of Extract solution 2 are added to the precipitate. The supernatant is broken by ultrasonic wave and centrifuged at  $4^{\circ}\text{C}$  for 10 min. The supernatant is diluted 2 times and detected by 96 well plate. The results showed that: cytoplasmic  $\Delta A_T = A_T - A_C = 0.377 - 0.34 = 0.037$ , mitochondrial  $\Delta A_T = A_T - A_C = 0.711 - 0.649 = 0.062$ , 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) =  $16.83 \times x \div W \times 2 = 1.55$  U/g mass, ICDHm activity in mitochondria (U/g mass) =  $6.73 \times x \div W \times 2 = 3.66$  U/g mass, total ICDHm (U/g mass) =  $16.83 \times x \div W \times 2 + 9.16 \times x \div W \times 2 = 5.21$  U/g mass.

#### Recent product Citations :

[1] Xiao Li, Qi Zhao, Gianni Qi, et al. lncRNA Ftx promotes aerobic glycolysis and tumor progression through the PPAR $\gamma$  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.

#### Related products :

- AK0282/AK0281  $\alpha$ -Ketoglutarate Dehydrogenase( $\alpha$ -KGDH) Activity Assay Kit
- AK0504/AK0503 Succinate Dehydrogenase(SDH) Activity Assay Kit
- AK0554/AK0553 Pyruvate Dehydrogenase(PDH) Activity Assay Kit