PRODUCT INFORMATION & MANUAL

NAD-Isocitrate Dehydrogenase/ NAD-IDH Activity Assay Kit (Colorimetric)

NBP3-25872

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

NAD-Isocitrate Dehydrogenase/NAD-IDH Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25872

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.84 U/L

Detection range: 0.84-50 U/L

Average intra-assay CV (%): 5.0

Average inter-assay CV (%): 8.6

Average recovery rate (%): 97

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure NAD-isocitrate dehydrogenase (NAD-IDH) activity in animal tissue samples.

▲Detection principle

Isocitrate dehydrogenase (IDH) is one of the inverters in the tricarboxylic acid cycle and plays an important role in energy metabolism, amino acid and vitamin synthesis. The cofactors of this enzyme include two kinds of NAD^+ and $NADP^+$, which are located in different parts of the cell respectively. In eukaryotic cells, NAD-dependent isocitrate dehydrogenase mainly exists in mitochondria.

Under the activation of the activator, IDH converts isocitrate into α-ketoglutaric acid. Meanwhile, NAD^+ is reduced to NADH, which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The activity of NAD-IDH can be calculated by measuring the change of absorbance value at 450 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Substrate	1.6 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Accelerant	powder × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 5	Standard	powder × 2 vials	-20℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Centrifuge, 37℃ incubator, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 with 1 mL double distilled water and mix fully. The prepared solution can be stored on the ice box for use. The remaining solution can be stored with shading light for 5 days at -20℃ , avoid repeated freezing-thawing.
3. Preparation of reaction working solution:
   Mix the reagent 1, reagent 2 and reagent 3 working solution at the ratio of 31:3:6 fully. The prepared solution can be stored on the ice box with shading light for use. The prepared solution should be used on the same day.
4. Preparation of 1 mmol/L standard solution:
   Dissolve a vial of reagent 5 with 1.6 mL double distilled water and mix fully. The prepared solution can be stored on the ice box for use. The remaining solution can be stored with shading light for 5 days at -20℃ , avoid repeated freezing-thawing.

▲ Sample preparation

Tissue sample:

Accurately weigh the tissue, add reagent 1 at a ratio of weight (g): volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.84-50 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Rat lung tissue homogenate	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	0.2	40	160
C	0.3	60	140
D	0.4	80	120
E	0.6	120	80
F	0.8	160	40
G	0.9	180	20
H	1	200	0

2. The measurement of samples

(1) Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

Control well: Add 10 μL of sample to the corresponding wells.

(2) Add 120 μL of reaction working solution to standard well and sample well.

Add 120 μL of reagent 1 to control well.

(3) Add 20 μL of reagent 4 to each well.

(4) Mix fully with microplate reader for 3 s.Then stand at room temperature with shading light for 5 min. Measure the OD value of each well at 450 nm with microplate reader, recorded as A_1. Incubate at 37℃ with shading light for 20 min, measure the OD value of each well at 450 nm with microplate reader, recorded as A_2, $\Delta A = A_1 - A_2$. Calculate the standard curve use the A_2 value.

▲Summary operation table

	Standard well	Sample well	Control well
Sample (μL)		10	10
Standard solution with different concentrations (μL)	10		
Reaction working solution (μL)	120	120	
Reagent 1 (μL)			120
Reagent 4 (μL)	20	20	20

Mix fully.Then stand at room temperature with shading light for 5 min. Measure the OD value of each well, recorded as A_1. Incubate at 37℃ with shading light for 20 min, measure the OD value of each well, recorded as A_2, $\Delta A = A_1 - A_2$. Calculate the standard curve use the A_2 value.

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

Tissue sample:

Definition: The amount of NAD-IDH in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{NAD-IDH activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0, the standard is calculated using only A_2 values).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{450}: $\Delta A_{Sample} - \Delta A_{Control}$ ($\Delta A = A_2 - A_1$).

1000: 1 mmol/L = 1000 μmol/L.

T: The time of reaction, 20 min

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For 10% mouse liver tissue homogenate, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.6007 x - 0.0051, The A_1 of the sample well is 0.457, the A_1 of the control well is 0.583. After 20 minutes of reaction, the A_2 of the sample well is 1.042, the A_2 of the control well is 0.841, $\Delta A_{Sample} = A_2 - A_1 = 0.585$, $\Delta A_{Control} = A_2 - A_1 = 0.258$, $\Delta A_{450} = 0.585 - 0.258 = 0.327$, the concentration of protein in sample is 8.34 gprot/L, and the calculation result is:

$$\text{NAD-IDH activity (U/gprot)} = (0.327 + 0.0051) \div 0.6007 \div 20 \times 1000 \div 8.34$$

$$= 3.31 \text{ U/gprot}$$