（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K651-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.84-50 U/L

Elabscience®NAD-Isocitrate Dehydrogenase (NAD-IDH) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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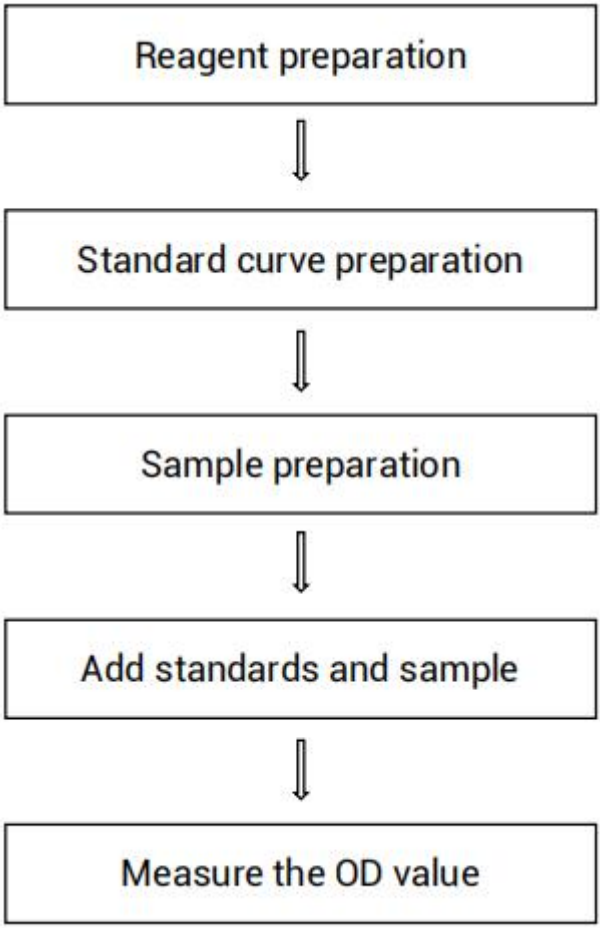
Assay summary



Intended use

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

Detection principle

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	Size (96 T)	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	
	Sample Layout Sheet	1 piece	

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of accelerant working solution：
Dissolve one vial of accelerant with 1 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 5 days protected from light, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of reaction working solution：
For each well, prepare 120 μL of reaction working solution (mix well 93 μL of buffer solution, 9 μL of substrate and 18 μL of accelerant working solution). Keep reaction working solution on ice during use and protect from light. The prepared solution should be used up within the same day.

④ The preparation of 1 mmol/L standard solution：
Dissolve one vial of standard with 1.6 mL of double distilled water, mix well to dissolve. Keep reaction working solution on ice during use. Aliquoted storage at -20°C for 5 days protected from light, and avoid repeated freeze/thaw cycles is advised.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.

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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1**
1 mmol/L standard (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

Operating steps

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 buffer solution to control well

③ Add 20 μL of chromogenic agent 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, $\triangle A = A_2 - A_1$, the standard is calculated using only A_2 values.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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℃ 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]

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

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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat kidney tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	18.40	38.50
%CV	5.4	4.9	4.7

Inter-assay Precision

Three rat kidney tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	18.40	38.50
%CV	8.2	9.4	8.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.25	0.52	0.88
Observed Conc. (mmol/L)	0.2	0.5	0.9
Recovery rate (%)	94	98	99

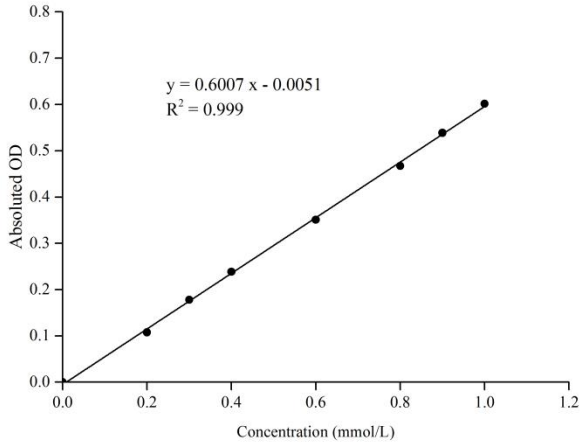
Sensitivity

The analytical sensitivity of the assay is 0.84 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1**
Average OD	0.077	0.185	0.255	0.316	0.428	0.544	0.616	0.679
Absoluted OD	0	0.108	0.178	0.239	0.351	0.467	0.539	0.602



Appendix Π Example Analysis

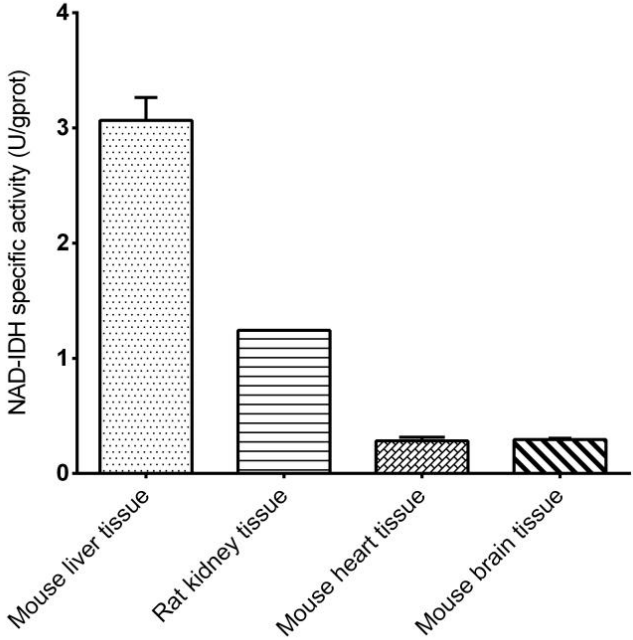
Example analysis：

For 10% mouse liver tissue homogenate, carry the assay according to the operation steps. 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, $\Delta A_{Sample} = A_2 - A_1 = 0.585$, $\Delta A_{Control} = A_2 - A_1 = 0.258$, $\triangle A_{450} = 0.585 - 0.258 = 0.327$, the concentration of protein in sample is 8.34 gprot/L, and the calculation result is:

NAD-IDH activity (U/gprot) = (0.327 + 0.0051) ÷ 0.6007 ÷ 20 × 1000 ÷ 8.34 = 3.31 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 8.34 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein is 4.96 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 5.34 gprot/L) and 10% mouse brain tissue homogenate (the concentration of protein is 3.56 gprot/L) according to the protocol, the result is as follows：


NAD-IDH specific activity (U/gprot)
4
3
2
1
0
Mouse liver tissue
Rat kidney tissue
Mouse heart tissue
Mouse brain tissue

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.