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

Catalog No：E-BC-K554-M

Specification：96T(80 samples)

Measuring instrument：Microplate reader(440-460 nm)

Detection range：0.10-50.47 U/L

Elabscience® NADP-Isocitrate Dehydrogenase (NADP-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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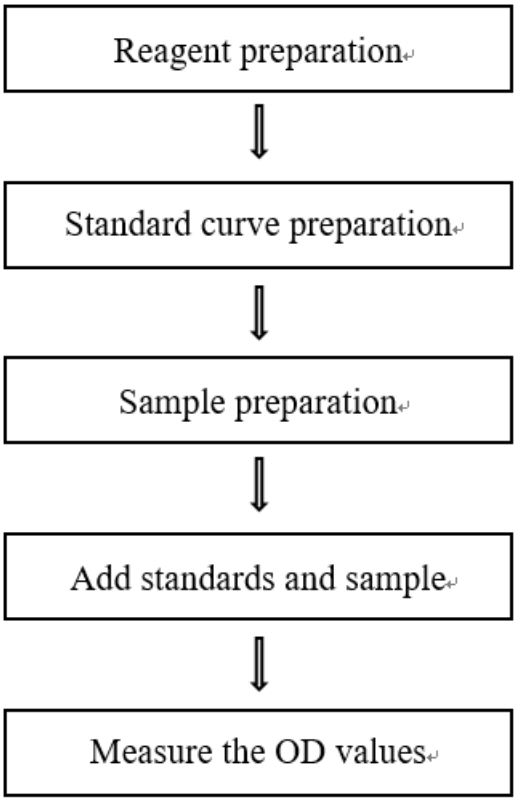
Assay summary



Intended use

This kit can be used to measure NADP-isocitrate dehydrogenase (NADP-IDH) activity in tissue and cell samples.

Detection principle

Isocitrate dehydrogenase (IDH) is one of the invertase in the tricarboxylic acid cycle, which plays an important role in energy metabolism and amino acid and vitamin synthesis. The cofactors of IDH include two types of NAD^+ and $NADP^+$, located in different parts of the cell. In eukaryotic cells, $NADP^+$ dependent isocitrate dehydrogenase is mainly present in the cytoplasm.

Isocitrate dehydrogenase converts isocitrate to α- ketoglutaric acid, while converting $NADP^+$ to NADPH. Under the action of electron coupling agents, the generated NADPH has a characteristic absorption peak at 450 nm. The activity of NADP-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	50mL ×2 vials	-20℃, 12 months, shading light
Reagent 2	Substrate	1.5 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Accelerant	1.5 mL ×1 vial	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent	2.5 mL ×1 vial	-20℃, 12 months, shading light
Reagent 5	10 mmol/L Standard	0.5 mL ×1 vial	-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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of reaction working solution:

For each well, prepare 120 μL of reaction working solution (mix well 102 μL of buffer solution, 9 μL of substrate and 9 μL of accelerant). Keep reaction working solution on ice protected from light during use. The prepared solution should be used up within 1 day.

③ Preparation of 0.5 mmol/L standard solution:

Dilute 50 μL of 10 mmol/L standard with 950 μL of double distilled water, mix well to dissolve. Keep 0.5 mmol/L standard solution on ice protected from light during use. The prepared solution should be used up within 8 hours.

④ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
0.5 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	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).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL buffer solution with a ultrasonic cell disruptor 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	10-60
10% Rat brain tissue homogenate	5-10
10% Rat liver tissue homogenate	10-80
1×10^6 CHO cells	1
1×10^6 RAW cells	1
1×10^6 293T cells	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Keep sample on ice during use.

Operating steps

① Standard well: add 20 μL of standards with different concentrations into the standard wells.

Sample well: add 20 μL of sample into the sample wells.

② Add 120 μL of reaction working solution to each well.

③ Add 20 μL of chromogenic agent to each well.

④ Mix well for 3 s with microplate reader. Incubate at 37℃ for 5 min protected from light.

⑤ Measure the OD value (A_1) of each well at 450 nm with microplate reader, then incubate at 37℃ for 20 min protected from light and measure the OD value (A_2) of each well at 450 nm. $\Delta A_{sample}=A_2-A_1$, $\Delta A_{450}=\Delta A_{sample}-\Delta A_{blank}$ (ΔA_{Blank} is the ΔA when the standard concentration is 0, $\Delta A_{Blank}= A_2-A_1$).

(Note: Standard wells only need to measure the OD values of A_2.)

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:

NADP-IDH activity in tissue and cell sample:

Definition: The amount of NADP-IDH in 1 g tissue or cell protein that catalyze the substrate to produce 1 μmol NADPH in 1 minute at 37 ℃ is defined as 1 unit.

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

[Note]

ΔA_{450}: $\Delta A_{450}=\Delta A_{sample}-\Delta A_{blank}$, ($\Delta A_{sample}=A_2-A_1$, ΔA_{Blank} is the ΔA when the standard concentration is 0).

T: The reaction time, 20 min.

1000： 1 mmol/L = 1000 μmol/L.

f: Dilution factor of sample before tested.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse lung 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)	0.88	1.49	2.26
%CV	5.00	6.00	6.00

Inter-assay Precision

Three mouse lung 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)	0.39	1.37	1.78
%CV	5.70	6.30	7.20

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 103%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	0.90	1.50	2.10
Observed Conc. (U/L)	0.88	1.49	2.26
Recovery rate (%)	97.00	100.00	111.00

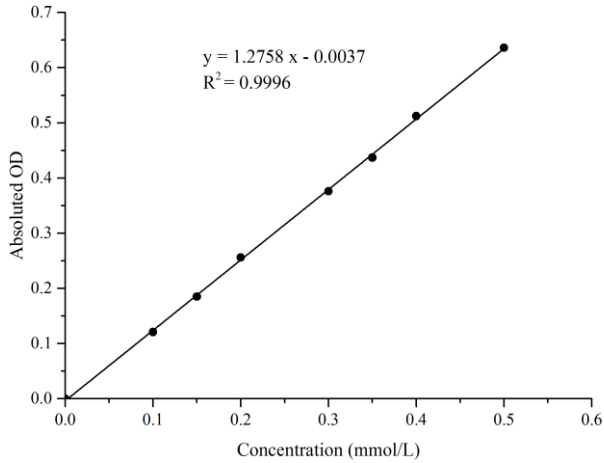
Sensitivity

The analytical sensitivity of the assay is 0.1 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**	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
OD value	0.056	0.172	0.235	0.310	0.432	0.486	0.563	0.691
	0.047	0.173	0.237	0.305	0.423	0.491	0.564	0.683
Average OD	0.052	0.173	0.236	0.308	0.428	0.489	0.564	0.687
Absoluted OD	0	0.121	0.185	0.256	0.376	0.437	0.512	0.636



Appendix II Example Analysis

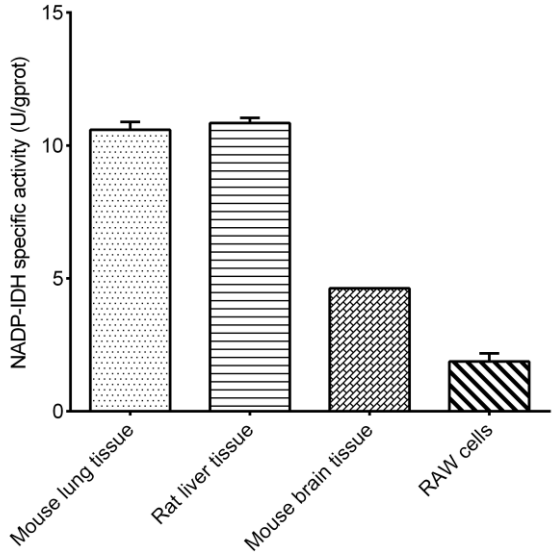
Example analysis:

For 10% mouse lung tissue homogenate, take 20 μL and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 1.2758 x- 0.0037, The A_1 of sample well is 0.391, the A_2 of sample well is 0.624. The ΔA of blank well is 0.005. ΔA_{450}= (0.624-0.391) - 0.005 = 0.228. The concentration of protein in sample is 8.77 gprot/L, and the calculation result is:

$$\text{NADP-IDH activity (U/gprot)} = (0.228 + 0.0037) \div 1.2758 \div 20 \times 1000 \div 8.77 \times 10 = 10.35 \text{ U/gprot}$$

Detect 10% mouse lung tissue homogenate (the concentration of protein is 8.77 gprot/L, dilute for 10 times), 10% rat liver tissue homogenate (the concentration of protein is 5.267 gprot/L, dilute for 10 times), 10% mouse brain tissue homogenate (the concentration of protein is 3.616 gprot/L, dilute for 10 times) and RAW cells (the concentration of protein is 0.182 gprot/L) according to the protocol, the result is as follows:



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.