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

Catalog No：E-BC-K196-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（550 nm）

Detection range：28.0-581 U/L

Elabscience® 5'-Nueleotidase (5'-NT) Activity Assay Kit

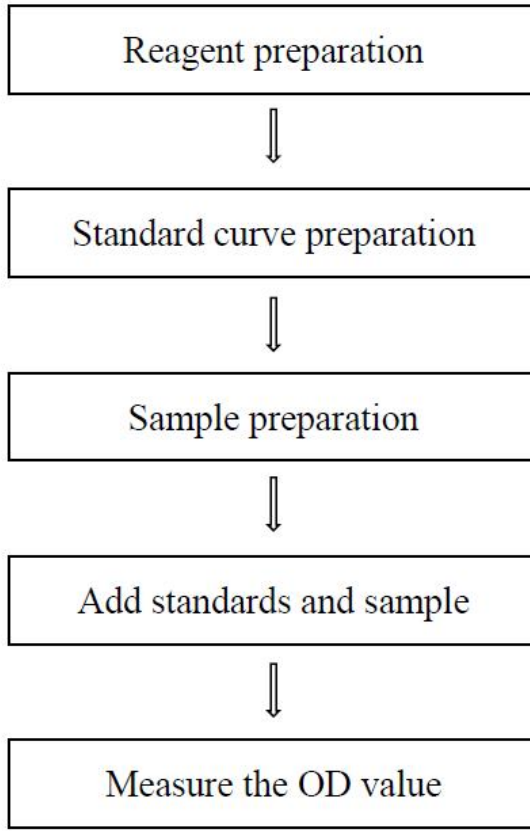
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure 5'-nueleotidase activity in serum, plasma and animal tissue samples.

Detection principle

5 '-NT hydrolyzes hypoxanthoside -5 '-monophosphate (5'-IMP) to produce inosine, which translates into hypoxanthine in the presence of purine nucleoside phosphorylase (PNP). Hypoxanthine translates into uric acid and H2O2 through xanthine oxidase, and H_2O_2 in the presence of peroxidase (POD) reacts with chromogen and 4-amino antipyrine (4 - APP) to produce colored quinone. The activity of 5 '-NT is calculated by measuring the increase rate of absorbance at 550 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent	5 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	0.6 mmol/L Inosine Standard Solution	2 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	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 (550 nm), Pipettor, Water bath, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard curve：

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

Dilute 0.6 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.12, 0.24, 0.30, 0.36, 0.42, 0.48, 0.60 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.12**	**0.24**	**0.30**	**0.36**	**0.42**	**0.48**	**0.60**
0.6 mmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation:

Serum (plasma): detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue samples:

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 normal saline (0.9% NaCl) with a dounce

homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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 brain tissue homogenate	1
10% Rat liver tissue homogenate	2-5
10% Rat lung tissue homogenate	1
10% Rat kidney tissue homogenate	2-5
Rat plasma	1
Dog serum	1
Human serum	1
Mouse plasma	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

② During incubation, the microplate should be with shading light.

③ When adding chromogenic agent, please add it to the wells as soon as quickly. The multichannel pipeter is recommended to shorten the time and reduce the error between wells.

Operating steps

① Standard well: add 10 μL of standard solution with different concentrations into the corresponding wells.

Sample well: add 10 μL of sample into the corresponding wells.

② Add 180 μL of working solution into the each well and incubate at 37°C for 5 min.

③ Add 90 μL of chromogenic agent into the each well.

④ Incubate at 37°C for 10 min. Measure the OD values of sample wells at 550 nm with microplate reader, recorded as A_1.

⑤ Incubate at 37°C for 10 min. Measure the OD values of each well at 550 nm with microplate reader, recorded as 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:

1. Serum (plasma) sample:

Unit definition: the enzyme amount of 1 μmol of inosine generated by 1 L of sample at 37°C for 10 minutes in the reaction system is defined as 1 unit.

$$\text{5'-NT activity (U/L)} = (A_2 - A_1 - b) \div a \times 1000^* \times f$$

2. Tissue sample:

Unit definition: the enzyme amount of 1 μmol of inosine generated by 1 g tissue protein at 37°C for 10 minutes in the reaction system is defined as 1 unit.

$$\text{5'-NT activity (U/gprot)} = (A_2 - A_1 - b) \div a \times 1000^* \div C_{pr}$$

[Note]

A_1: The absorbance of the samples at the first time of incubation for 10 min;

A_2: The absorbance of the samples at the second time of incubation for 10 min;

1000*: 1 mmol=1000 μmol;

f: Dilution factor of sample before test;

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	52.00	227.00	406.00
%CV	3.8	3.4	2.7

Inter-assay Precision

Three human serum 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)	52.00	227.00	406.00
%CV	6.1	6.3	5.6

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 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.21	0.34	0.45
Observed Conc. (mmol/L)	0.2	0.4	0.5
Recovery rate (%)	108	103	104

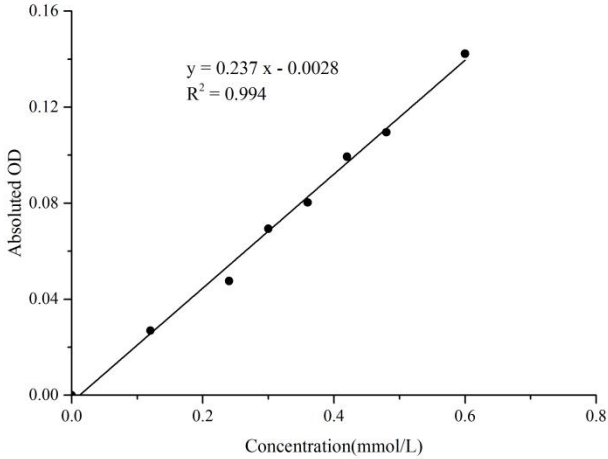
Sensitivity

The analytical sensitivity of the assay is 28.0 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)	0.00	0.12	0.24	0.30	0.36	0.42	0.48	0.60
OD value	0.046	0.073	0.100	0.116	0.126	0.143	0.153	0.192
	0.045	0.072	0.087	0.115	0.126	0.147	0.158	0.184
Average OD	0.046	0.073	0.093	0.115	0.126	0.145	0.155	0.188
Absoluted OD	0.000	0.027	0.048	0.069	0.080	0.099	0.110	0.142



Appendix II Example Analysis

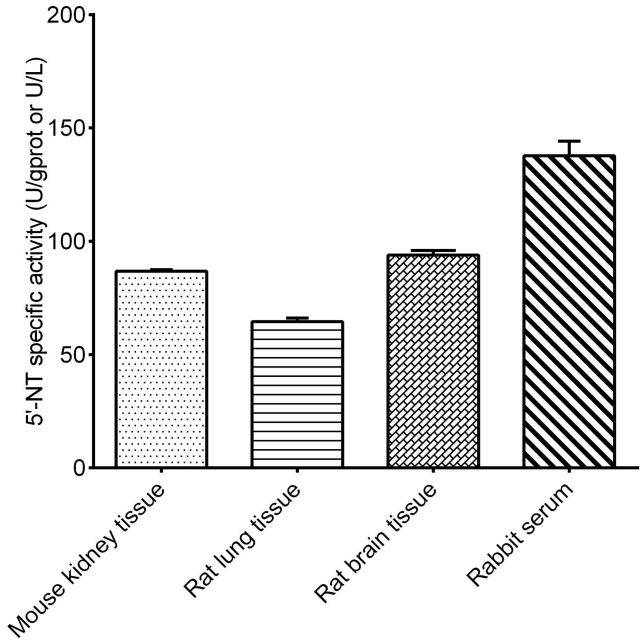
Example analysis:

Take 5 μL of 10% mouse kidney tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.298 x – 0.0034, the first incubation time for 10 min, the average OD value of the sample (A_1) is 0.193, the second incubation time for 10 min, the average OD value of the sample (A_2) is 0.307, the concentration of protein in sample is 4.54 gprot/L, and the calculation result is:

5 '-NT content (U/gprot) = (0.307 - 0.193 + 0.0034) ÷ 0.298 ÷ 4.54 × 1000 = 86.77 U/gprot

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 4.54 gprot/L), 10% rat lung tissue homogenate (the concentration of protein is 6.35 gprot/L), 10% rat brain tissue homogenate (the concentration of protein is 3.11 gprot/L) and rabbit serum 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.