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

Catalog No: E-BC-K196-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 28.0 U/L

Detection range: 28.0-581 U/L

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 glutamic acid content in serum, plasma and animal tissue samples.

▲Background

5'-Nucleotide enzyme (5'-NT, EC 3.1.3.5), full name is 5'-ribonucleotide phosphohydrolase, is a special phosphohydrolase that specifically hydrolyzes 5'-phosphoric acid attached to pentose in 5'-nucleotide. This enzyme is widely distributed in the cell membrane of various tissues of human and animal. Only the 5'-NT released by the tissue cells of the hepatobiliary system may enter the blood. Therefore, the source of serum 5'-NT has certain specificity, and the determination of serum 5'-NT has important value for the diagnosis of hepatobiliary diseases.

▲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 H_2O_2 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	Specification	Storage
Reagent 1	Working Solution	20 mL × 1 vial	2-8°C, 6 months, shading light
Reagent 2	Chromogenic Agent	10 mL × 1 vial	2-8°C, 6 months, shading light
Reagent 3	0.6 mmol/L Inosine Standard Solution	2 mL × 1 vial	2-8°C, 6 months
	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

Microplate reader (550 nm), Pipettor, Water bath, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲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.

▲The key point 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 reagent 2, 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.

Pre-assay preparation

▲Reagent preparation

Bring all the reagents to room temperature before use.

▲Sample preparation

1. Serum (Plasma): Detect directly.
2. 10% Tissue homogenate: Accurately weigh the tissue sample, add normal saline (0.9% NaCl) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲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 (28.0-581 U/L).

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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	550 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

1.The preparation of standard curve

Dilute 0.6 mmol/L standard solution with double distilled water 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.

2.The measurement of sample

1) Standard weii: 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 reagent 1 into the each well and incubate at 37°C for 5 min.
3) Add 90 μL of reagent 2 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.

▲Operation table

	Standard well	Sample well
Sample (μL)	10	
Standards solution with different concentrations (μL)		10
Reagent 1 (μL)	180	180
Incubate at 37°C for 5 min		
Reagent 2 (μL)	90	90
Incubate at 37°C for 10 min. Measure the OD values of sample wells at 550 nm with microplate reader, recorded as A_1.		
Incubate at 37℃ for 10 min. Measure the OD values of each well at 550 nm with microplate reader, recorded as A_2.		

Note: Standard well detects A_2, while sample well detects A_1 and A_2.

▲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: $y = ax + b$.

1. For serum (plasma) samples

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.

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

2. For 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.

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

Note:

y: $OD_{Standard}$ - OD_{Blank} (OD_{Blank} is the change of OD value when the standard concentration is 0);

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve;

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}: Concentration of the protein in sample, gprot/L.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	28.0-581 U/L	Average intra-assay CV (%)	3.3
Sensitivity	28.0 U/L	Average inter-assay CV (%)	6.0
Average recovery rate (%)	105		

▲Example analysis

Take 5 μL of 10% mouse kidney tissue homogenate and carry the assay according to the operation table. 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:

$$5'\text{-NT content (U/gprot)} = (0.307-0.193+0.0034) \div 0.298 \div 4.54 \times 1000 = 86.77\ \text{U/gprot}$$