Technical Manual

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

- Catalogue Code: MAES0140
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

28.0-581 U/L

Sensitivity:

28.0 U/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background:

5'-Nucleotide enzyme (5'-NT, EC 3.1.3.5), full name 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 humans and animals. 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 an important value for the diagnosis of hepatobiliary diseases.

3. Intended Use:

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

4. 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.

5. Kit Components & Storage:

Item	Specification	Storage
Working Solution	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent	10 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Inosine Standard Solution (0.6 mmol/L)	2 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (550 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

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

2. During incubation, the microplate should avoid direct sunlight.

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

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation:

1. Serum (Plasma): Detect directly.

2. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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);

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 550 nm

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.

10. Operation Steps:

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.

The measurement of samples

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.

Operation Table

	Standard well	Sample well
Sample (µL)		10
Standards solution with different concentrations (µL)	10	
Working Solution (µL)	180	180
Incubate at 37°C for 5 min		
Chromogenic agent (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°C for 10 min. Measure the OD values of each well at 550 nm with microplate reader, recorded as A_2.		

11. Calculations:

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. 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 and cells 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.

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the 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 protein in sample, gprot/L

12. Performance Characteristics:

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

Analysis

Take 10 µL of 10% mouse kidney tissue homogenate, 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:

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

$$= 86.77 \text{ (U/gprot)}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

Email: info@assaygenie.com **Web:** www.assaygenie.com

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