TECHNICAL MANUAL

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

- **SKU CODE:** MAES0140
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and add chromogenic agent
- Measure absorbance values
- Calculate enzyme activity

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	10 mL x 1 vial	20 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 2	Chromogenic Agent	5 mL x 1 vial	10 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 3	0.6 mmol/L Inosine Standard Solution	2 mL x 1 vial	2 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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 to create 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.

7. Sample preparation

Sample preparation:

Serum (plasma): Detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for one 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 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of samples:

The recommended dilution factor for different samples is as follows (for reference only):

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 dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. Key points of the assay

1. Prevent the formation of bubbles when transferring supernatant into the microplate.
2. During incubation, the microplate should be protected from light.
3. When adding chromogenic agent, add it to the wells as quickly as possible. A multichannel pipettor is recommended to shorten the time and reduce error between wells.

9. Operating steps

1. Standard wells: Add 10 μL of standard solution with different concentrations into the corresponding wells. Sample wells: Add 10 μL of sample into the corresponding wells.
2. Add 180 μL of working solution into each well and incubate at 37 °C for 5 min.
3. Add 90 μL of chromogenic agent into 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.

10. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding 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 that generates 1 μmol of inosine per 1 L of sample at 37 °C for 10 minutes in the reaction system is defined as 1 unit.

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

2. Tissue sample:

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

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

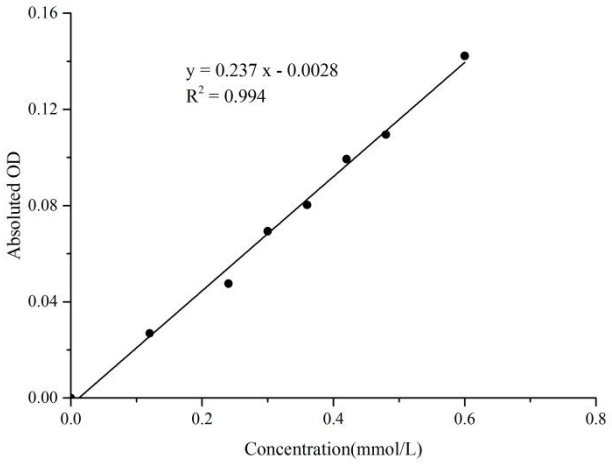
Three samples of high, middle, and low concentration were tested with 6 replicates for each concentration to obtain an average recovery rate of 105%.

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.

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), the standard curve and data are provided below for reference only.



12. Appendix II Example Analysis

Example analysis:

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

Standard curve: y = 0.298x - 0.0034

First incubation time for 10 min: average OD value of the sample (A_1) is 0.193

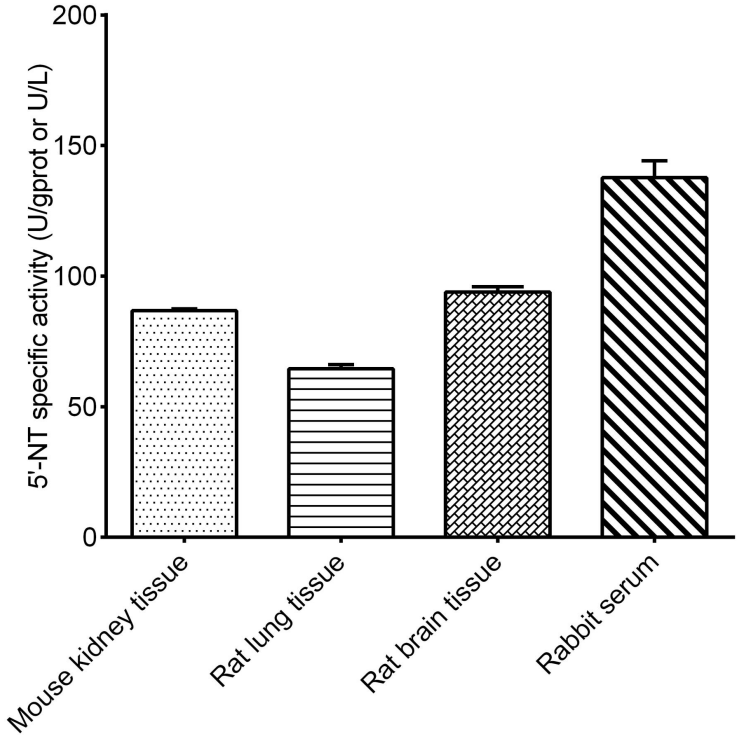
Second incubation time for 10 min: average OD value of the sample (A_2) is 0.307

Protein concentration in sample: 4.54 gprot/L

Calculation result:

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 (protein concentration: 4.54 gprot/L), 10% rat lung tissue homogenate (protein concentration: 6.35 gprot/L), 10% rat brain tissue homogenate (protein concentration: 3.11 gprot/L), and rabbit serum according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.