

Instructions for Use

Version: 1.0.1

Revision date: 2-Sep-26



5'-Nucleotidase (5'-NT) Assay Kit

Catalog No.: abx294121

Size: 96 tests

Detection Range: 28 U/L – 581 U/L

Sensitivity: 28 U/L

Storage: Store the components in the dark at 4°C.

Application: For detection and quantification of 5'-nucleotidase activity in serum, plasma, and tissue homogenates.

Principle of the Assay

Abbexa's 5'-Nucleotidase (5'-NT) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating 5'-NT activity. 5'-NT hydrolyzes hypoxanthoside-5'-monophosphate (5'-IMP) to produce inosine, which is converted into hypoxanthine in the presence of purine nucleoside phosphorylase (PNP). Xanthine oxidase turns hypoxanthine into uric acid and hydrogen peroxide. Peroxidase (POD) catalyzes the reaction between hydrogen peroxide, Chromogenic Reagent and 4-amino antipyrine (APP) to produce a colored quinone with an absorbance of 550 nm. The absorbance at 550 nm is proportional to the 5'-NT activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Working Solution: 20 ml
3. Chromogenic Reagent: 10 ml
4. Standard (0.6 mmol/L): 2 ml
5. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (550 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vials/tubes
7. Centrifuge
8. Vortex mixer

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Standards:** Label 7 tubes with 0.60 mmol/L, 0.48 mmol/L, 0.42 mmol/L, 0.36 mmol/L, 0.30 mmol/L, 0.24 mmol/L, and 0.12 mmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (mmol/L)	0.12	0.24	0.30	0.36	0.42	0.48	0.60
0.6 mmol/L Standard (μl)	40	80	100	120	140	160	200
Double-distilled water (μl)	160	120	100	80	60	40	0

For the blank, use pure double-distilled water. The volume of each standard dilution will be 200 μl.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and plasma:** Serum and plasma samples can be tested directly. Prepared sample can be aliquoted and stored at -80°C for up to 1 month
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue. Wash the tissue with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 μl of cold normal saline (0.9% NaCl). Homogenize manually or use a mechanical homogenizer at 4°C. Centrifuge at 10000 × g for 10 minutes. Carefully take the supernatant for detection. Keep on ice and assay immediately.

Note: To calculate 5'-NT concentration in tissue homogenates using the formulae in section C. **Calculation of Results**, the total protein concentration of the supernatant must be determined separately (abx097193).

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure.

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

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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:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

B. Assay Procedure

- Set standard and sample wells on the microplate and record their positions. Standards and samples should be run in duplicate. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
- Add 10 µl of each standard dilution to the standard wells.
- Add 10 µl of sample to the sample wells.
- Add 180 µl of Working Solution to each well.
- Cover with a plate sealer, then incubate at 37°C for 5 minutes
- Add 90 µl of Chromogenic Reagent to each well.
- Incubate at 37°C for 10 minutes. Measure the OD values of the wells at 550 nm with a microplate reader and record this value as A₁.
- Incubate at 37°C for an additional 10 minutes. Measure the OD values of the wells at 550 nm with a microplate reader and record this value as A₂.

C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y=ax+b$.

Based on this curve, the activity of 5'-NT in each sample well can be derived using the following formulae:

1. Serum and plasma samples: One unit of 5'-NT activity is defined as the amount of enzyme in 1 L of sample required to produce 1 µmol of inosine in 10 minutes at 37°C.

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$$5'\text{-NT activity (U/L)} = \frac{(A_2 - A_1 - b)}{a} \times F \times 1000$$

2. Tissues and cell lysate samples: One unit of 5'-NT activity is defined as the amount of enzyme in 1 g of tissue protein required to produce 1 μmol of inosine in 10 minutes at 37°C.

$$5'\text{-NT activity (U/g protein)} = \frac{(A_2 - A_1 - b)}{a \times C_{\text{Protein}}} \times 1000$$

where:

A_1	Absorbance of samples measured after the first 10-minute incubation period
A_2	Absorbance of samples measured after the second 10-minute incubation period
C_{Protein}	Concentration of protein in sample (g/L)
1000	Conversion factor from mmol to μmol (1 mmol = 1000 μmol)
F	Dilution factor of sample before test
a	Gradient of the standard curve ($y=ax+b$)
b	Y-intercept of the standard curve ($y=ax+b$)

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.