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

Method: Peroxidase

Cat .No.	Size	Instrument
GB8010G	R1: 2 × 20 ml R2: 1 × 20 ml	For Hitachi 717 & ShimadzuCL7200/8000
GS8011G	R1: 2 × 20 ml R2: 1 × 20 ml	For Hitachi917 & OlympusAU640/400/600
GX8011G	R1: 2 × 20 ml R2: 1 × 20 ml	For SYNCHRON CX4-5-7-9/LX20

INTENDED USE

For the *in vitro* quantitative determination of 5'-nucleotidase activity in human serum or plasma.

CLINICAL SIGNIFICANCE

5'-NT is an enzyme catalyzing the hydrolysis of nucleoside-5'-monophosphates to nucleosides and inorganic phosphate. The enzyme is widely distributed in human and animal tissues. The activity present in sera is released from the membrane of liver cells by bile salts and has been used as a marker for liver disease[1]. Increased enzyme levels in sera are associated with certain forms of liver disease, such as intra or extra-hepatic obstruction and particularly in cases of hepatic carcinoma as well as in mastectomy patients with recurrent metastases. The diagnostic value of 5'-NT has been shown to be superior to other liver enzymes, especially in cases of liver metastasis .

ASSAY PRINCIPLE

The 5'-NT assay is based on the enzymatic hydrolysis of 5'-monophosphate (5'-IMP) to form inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

$$IMP + H_2O \xrightarrow{5'\text{-NT}} Inosine + Pi$$

$$Inosine + Pi \xrightarrow{PNP} Hypoxanthine + Ribose\ 1\text{-}phosphate$$

$$Hypoxanthine + 2H_2O + 2O_2 \xrightarrow{XOD} Uric\ acid + 2H_2O_2$$

$$2H_2O_2 + 4\text{-}AA + EHSPT \xrightarrow{POD} 4H_2O + Quinone\ dye$$

(λ_{max}=556 nm)

SAMPLE COLLECTION AND PREPARATION

Serum or plasma samples.

Use fresh and non-hemolyzed serum or EDTA treated plasma samples. Serum or plasma samples are stable for a week at 4°C.

REAGENT COMPOSITION

Contents	Concentration of Solutions
Reagent 1 (R1)	
Tris	50 mM
IMP	10 mM
EHSPT	2 mM
Stabilizer	
Reagent 2 (R2)	
Tris	50 mM
4-AA	2 mM
PNP	0.1 U/L
XOD	0.2 U/L
POD	0.6 U/L
Stabilizer	

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.

Stable up to the expiry date when stored at 2-8℃.

The assay kit reagents are stable for 1 month after opening and kept at 2-8℃.

ASSAY PROCEDURE

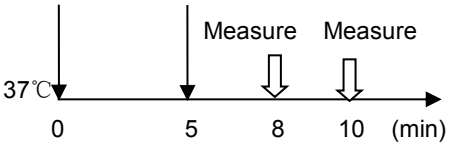
Test Procedure for Analyzers (HITACHI 7170/917)

Assay Mode: 2 Point Rate 28-34

Wave Length (main/sub): 546 nm/800 nm

Sample: 5 µl

R1: 180 µl R2: 90 µl



1. Mix 5 µl sample with 180 µl R1 and incubate at 37℃ for 5 minutes.
2. Add 90 µl R2 into cuvette, mix and incubate for 3 minutes at 37℃.
3. Read initial absorbance and start timer simultaneously, read again after 1 and 2 minutes.
4. Calculate absorbance change per minute (ΔA/min)

CALCULATION (ε=16.18)

$$5'\text{-NT (U/L)} = \frac{\Delta A/min \times V_t}{\varepsilon \times V_s \times L} \Delta A/min \times 3400$$

QUALITY CONTROL

For quality control, use 5'-NT control as daily quality control sera and can be purchased separately. Values should fall within a specific range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

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REFERENCE VALUE

Serum or plasma: 0-10 U/L
It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY

The method is linear up to 300 U/L. Samples above this concentration should be diluted 1+1 with 0.9% NaCl and reassayed. Multiply the result by 2.

PRECISION

The CV of the test should be ≤10%.

Intra assay precision		
N=20	Level1	Level 2
Mean	7.5	78.8
SD	0.11	0.95
CV	1.5%	1.2%
Inter assay precision		
N=5	Level1	Level 2
Mean	7.83	79.3
SD	0.16	1.35
CV	2.0%	1.7%

SENSITIVITY

The minimum detectable level that can be distinguished from zero has been determined as 1.81 U/L.

INTERFERENCE

Hemoglobin≤ 500 mg/dl, Intralipid ≤1000 mg/dl
ALP≤1250 U/l, without interference.
Bilirubin≤40 mg/dl, Ascorbic Acid≤50 mg/dl, interference.

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
Y=1.0426X+1.3074, R^2=0.9912; 64 patient samples were analyzed .

SAFETY PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. Reagents contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES

1. Eroglu A. Activities of adenosine deaminase and 5' - nucleotidase in cancerous and noncancerous human cdorectal tissues[J]. Med Oneol 2000; 17(4) :319 - 24.1
2. Alain Bertrand and Jean Buret, A one-step determination of 5'-nucleotidase using a centrifugal analyzer. Clinica Chimica Acta,119(1982)275-284.
3. By Z. Ahmed and J.L.Reis, The Activation and Inhibition of 5-Nucleotidase. Clin Chem. 1998，69（11），102－106.

INDEX OF SYMBOLS

	Manufacture
REF	Catalogue Number
LOT	Lot number
	Date of manufacture
	Use by(Expiration date)
IVD	For In-Vitro Diagnostic use only
2℃ 8℃	Stored at 2-8℃
	Attention:See instruction for use
EC REP	Authorized Representative in the European Company

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