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5' – Nucleotidase (5'-NT)
Catalog Number: BQ013-EALD

Method: Colorimetric Assay (Kinetic)
Wavelength: 550 nm
Linear Range: 0-300 U/L

Intended Use
5'-Nucleotidase (5'-NT) assay kit is for determination of 5'-NT activity in human serum samples.

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 (1-6).

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 (H2O2) by xanthine oxidase (XOD). H2O2 is further reacted with NEthyl-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.

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

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

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

$$2H_2O_2 + 4\text{-}AA + EHSPT \xrightarrow{POD} 4H_2O + Quinone\ dye\ (\lambda\ max\ 550nm)$$

One unit of 5'-NT is defined as the amount of 5'-NT that generates one µmole of inosine from IMP per min at 37°C.

Reagent Composition (275 tests)

Reagent 1 (R1) 50 mL	100 mM Goods buffer pH 7.6 2 mM 4-AA 0.1 U/mL PNP 0.2 U/mL XO 0.6 U/mL Peroxidase Stablizers
Reagent 2 (R2) 25 mL	100 mM Goods buffer pH 7.6 10 mM 5'-inosine monophosphate 2 mM EHSPT
5'-NT Control 1.0 mL	5'-nucleotidase Control is sold separately. BQ013-EACN

Reagent Preparation
Reagents are ready-to-use and stable for 9 months when stored at 2 – 8 °C.

5'-NT Control (sold separately) is provided in a lyophilized form, and needs to be reconstituted with 1.0 mL of DI water. After reconstitution, control vials should be equilibrated overnight at 2-8 °C. Reconstituted 5'-NT controls are stable for 1 week at 2-8°C.

Reagent Stability and Storage
The assay reagents are stable up to the expiration date on the label when stored at 2-8 °C. R1 is light sensitive. Reconstituted controls are stable for one week at 2-8 °C.

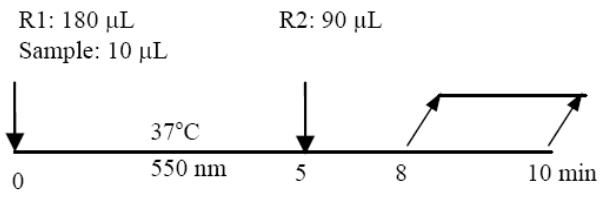
Materials Required But Not Provided
An analyzer capable of dispensing two reagents and of measuring absorbance at 550nm with temperature control (37 °C).

Sample Collection and Handling
Use fresh and non-hemolyzed serum or plasma for 5'-NT test. 5'-NT is stable in serum for one week at 2-8 °C.

Assay Procedure

- Reagents R1 and R2 are equilibrated to room temperature use in the assay. R1 is light sensitivity.
- Mix 180 µL of R1and 10 µL of plasma sample.
- Incubate at 37 °C for 5 min.
- Add 90 µL of R2, and incubate for 3 min followed by monitoring the absorbance at 550 nm for the last 2 min with 1 min interval to obtain ΔA/min values.

Assay procedure for chemistry analyzers is depicted in the scheme shown below:



Assay by Factor Method

- Calculate the average rate of the absorbance change ΔA/min.

$$\Delta A/min = \frac{(\Delta A_1/min + \Delta A_2/min)}{2}$$

- Calculate 5'-NT activity (U/L) in the plasma sample by using the formula:

$$5'\text{-}NT\ (U/L) = \frac{\Delta A/min.\ x\ Tv}{\varepsilon\ x\ Sv\ x\ L} = \Delta A/min\ x\ \mathbf{1518}$$

Note:
Before performing the assay in lab instrument or analyzer, users should verify the accuracy of the calculation factor. The calculation Factor for UV spectrophotometer is **1518** when the cuvette path length is 1 cm.
Users should determine the calculation factor for the specific instrument being used in the lab based on cuvette pathlength and other conditions. This can be done experimentally as follows:

1) Bio-Quant controls with known values are run in triplicate
2) The calculation factor is modified so that the result matches Bio-Quant control target values.
3) Bio-Quant 5'-NT controls can be purchased separately.

ε: µmolar extinction coefficient of quinone dye
($\varepsilon = 18.44 \times 10^{-3}$ µM-1cm^{-1})
Tv: Total reaction volume (mL)
Sv: Sample volume (mL)
L: Cuvette light path length (1.0 cm)

Calibration
A single calibrator is needed for running the assay in calibration mode. The calibrator is provided separately. Alternatively, a factor of 1518 is used for calculating the activity.

Quality Control
Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guideline concerning the running of external quality control.
To ensure adequate quality control, normal and abnormal control with known values should be run as unknown samples.
Controls are sold separately.

Results
The assay is run using calibrators or alternatively by use of a factor for calculating activity.

Reference Range
Healthy subjects have a 5'-NT activity in the range of 0 – 10 U/L. It is recommended that each laboratory should establish its own range of reference values.

Limitations
If the sample 5'-NT activity is greater than 300 U/L, the sample should be diluted with saline before measurement. The result should be multiplied by the dilution factor.

Performance Characteristics

Precision
The precision of the 5' Nucleotidase assay was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study within-run precision was determined by running fifteen (15) replicates of two (2) serum specimens with activities of 43.0 and 87.1 U/L of 5'-NT in one run.

Within-Run Precision	Level 1	Level 2
Mean (U/L)	43.0	87.1
SD	1.30	0.71
CV (%)	1.49	1.65

Between run precision was determined by running two replicates each of two serum samples on ten different days.

Between -Run Precision	Level 1	Level 2
Mean (U/L)	44.1	85.6
SD	1.46	3.43
CV (%)	3.31	4.00

Assay Linearity
The assay is linear from 0-300 U/L (r^2 >0.99).

Interference
Assay is not affected by serum bilirubin up to 40mg/dL, hemoglobin up to 500 mg/dL, triglycerides up to 1250mg/dL, and ascorbic acid up to 20 mg/dL, alkaline phosphatase up to 1250u/L.

Safety Precautions and Warnings
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.

Reagent 1 (R1) contains 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.

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.

References

1. Goldberg, D. M., Digestion 8, 87-99 (1973)
2. Drummond, G. I. & Masanobu, Y. In: The enzymes (boyer, P.D. (ed.), (3rd ed.), vol. 4, pp. 337 (1971)
3. Kim, N. K., Yasmineh, W. G., Treier, E. F., Goldman, A. & Theologides, A. Clin. Chem. 23, 2034-2038 (1977)
4. Van der Hik, W., Persijn, J. P. et al. Clin. Biochem. 3: 59-80 (1970)
5. Heinz, F., Pilz, R., Reckel S.,Kalden JR., & Haeckel R. J. Clin. Chem. Clin. Biochem. 18: 781-788 (1980)
6. Bertrand A. and Buret J. Clin. Chimi. Acta 119: 275-284 (1982)

Cobas Mira Parameters	**5'-NT BQ013EALD**
Measurement Mode	Absorb
Reaction Mode	R-S-SR1
Calibration Mode	Factor
Reagent Blank	Reag/DIL
Cleaner	No
Wavelength	550 nm
Decimal Position	2
Unit	U/L
Sample cycle	1
Sample volume	10 uL
Sample dilution	H_2O
Dilution volume	0.0 uL
Reagent cycle	1
Reagent volume	180 uL
Dilution volume	0.0 uL
Start R1 cycle	12
Reagent volume	90 ul
Dilution volume	0.0 uL
Sample limit	No
Reaction direction	Increase
Convers. factor	1.0000
Offset	0.0000
Test range low	0.000 U/L
Test range High	300.00 U/L
Number of steps 1	1
Calc. Step A	Kinetics
Readings first	19*
Readings last	24*
Calibration	
Cali. interval	Each Run
Blank	
Reagent range low	-0.1
high	0.3
Blank range low	-0.1
high	0.1
Factor	3036

* Each reading cycle is 25 seconds.

HITACHI 717 Parameters	5'-NT BQ013EALD
Test	5'-NT
Assay Code	Rate-A
Assay Point	(39)-(49)**
Wavelength	700/546
Calibration Method	K Factor
STD. (1) CONC.-Position	(0)-(1)* (blank)
Unit	U/L
Sample volume	(10)(10)
Reagent vol. R1	(180)(100)(NO)
Reagent vol. R2	(90)(100)(NO)
K Factor	1940
ABS Limit	32000-Increse
Expected value (normal Value)	0-10 U/L
Tech. Limit	0-300

Attention: * Entered By Operator
** Each reading cycle is 12 seconds.

5'-Nucleotidase assay procedure for Tecan Plate Reader

1. Prepare a dilution series of calibrators.
   Use 1.0 ml DI water to reconstitute control vial (concentration is 120 U/L). Then use DI water to set up a 1:2 dilution series (60, 30, 15 and 7.5 U/L). DI water is used as a blank.
2. Pipette 10 µl of DI water (blank), calibrator or sample into plate wells.
3. Pipette 180 µl Reagent 1 into each well containing DI water (blank), calibrator or sample.
4. Incubate for 5 min at 37 °C on the Tecan Reader (Step 1).
5. Pipette 90 µl Reagent 2 into each well containing DI water (blank), calibrator or sample with a multi-pipette as quickly as possible.
6. Incubate for 3 min at 37 °C, and read for a further 3 min on Tecan at 37 °C. (Step 2).

Measurement Parameters on TECAN:

Step 1.

Measurement mode:	Absorbance
Measurement wavelength:	550nm
Incubation time:	290 s
Shake duration:	10 s

Step 2.

Measurement mode:	Absorbance
Measurement wavelength:	550nm
Read mode:	Accuracy
Number of kinetic cycles:	12
Kinetic interval:	15 s
Valid temperature range:	36-38 °C
Incubation time:	170 s
Shake duration (inside normal):	10 s
Unit:	OD

Instrument Parameters for Olympus AU 400

Temperature 37 °C

General

Test Name: 5NT Type: Serum Operation: Yes

Sample Volume 10.0 µL Dilution 0 µL Pr-Dilution Rate 1

Reagents: Min OD Max OD

R1 volume 180 µL Dilution 0 µL L: -2.000 H: 2.500

R2 volume 90 µL Dilution 0 µL

Wavelength: Pri. 540 Sec. 700 Reagent OD Limit:

Method: Rate First L:-2.000; First H: 2.500

Reaction Slope: + Last L: -2.000; Last H: 2.500

Measuring Point 1: First 20; Last 27 Dynamic Range:

Measuring Point 2: First ; Last L: 0.00 H: 999.0

Linearity 20% Correlation Factor:

No-Lag-Time: No A: 1.0000 B: 0.0000

Onboard stability Period: 999

Calibration Type MB Formula: Y=AX+B

Counts 2 Process

Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1				
Point 2				

Advanced Cal.ibration: No

MB Type Factor: **2450.000** Calibration Stability Period: 999