

5'-NUCLEOTIDASE (5'ND, NTP)

KINETIC UV DETERMINATION IN SERUM
 For in vitro diagnostic use only

Kit: 10 x 6.5 mL

Cod. NU8860

SUMMARY

5' Nucleotidase (NTP) determinations are valuable in differentiating between elevations in ALP (Alkaline Phosphatase) caused by hepatobiliary diseases and those caused by disease involving the skeletal system.

In fact any rise in NTP that is significant is virtually specific for hepatobiliary disease; NTP in the serum increase 2-6 fold in those hepatobiliary diseases in which there is interference with the secretion of the bile.

This may be due to intrahepatic conditions, as cholestasis caused by biliary cirrhosis, malignant infiltration of the liver, chlorpromazine; or it may be due to extrahepatic conditions, as tumor occluding the bile duct or a stone.

PRINCIPLE

The 5'-Nucleotide (5'ND) catalyzes the hydrolysis of AMP to Adenosine; this one is deaminated by Adenosine Deaminase (ADA), giving inosine and Ammonium ion that in the presence of Glutamate dehydrogenase (GIDH), 2-oxo-glutarate and NADH forms glutamate and NAD.

The decrease of absorbance of NADH, for oxidation to NAD, is proportional to the activity of the 5'-Nucleotidase in the sample.

REAGENTS

Components of the kit:	Cod. NU8860
*REAGENT 1 (liquid)	1 x 65 mL
*REAGENT 2 (lyo)	10 x 6.5 mL
Good Buffer	>20 mmol/L
AMP	>2.5 mmol/L
NADH	>0.16 mmol/L
2-Oxoglutarate	>2.5 mmol/L
GIDH	>6 KU/L
ADA	>0.3 KU/L
Activators, Inhibitor, Stabilizers	
*REAGENT 3 (lyo)-CALIBRATOR	1 x 3 mL
5'-Nucleotidase (value in U/l met.UV at 37°C on the label)	

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package if **not contaminated during handling**.

AUXILIARY REAGENTS (Not supplied with this kit)

To assure proper test performance, we suggest following kits:
 - **LEVEL 1 ENZYME Control 5 x 3 mL** Cod. BEE1005
 that contains a few enzymes (see the inserts).

PREPARAT. OF THE WORKING REAGENT (*R1+*R2)

*KIT 10 x 6.5 ml (Cod. NU8860)

Add 6.5 ml of *Reagent 1 to one vial of *Reagent 2. Mix gently until dissolution.

A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

STABILITY: 2 days at 2-8°C; 10 days if frozen at -20°C.

FREEZE only ONE TIME. DO NOT REPEAT FREEZING.

Mix kindly and let the working reagent reaches the working temperature before use. Close immediately after handling.

The Reagents have to be used properly, to avoid contamination. Incompetent handling will keep us harmless from any responsibility.

PREPARATION OF CALIBRATOR (*R3)

Add 3.0 ml of dist. water; close bottle carefully and let stand for 30 min. Mix gently until dissolution.

A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

STABILITY: at -20° C at least 1 month, when frozen once.
 at +2-8° C at least 5 days.

CAUTION

The Calibrator is from human origin and has to be used as a potential transfer of infective pathologies. This product has been tested and found to be negative for HIV, HCV and HBsAg antibodies by an approved method. Because no test method can offer complete assurance that all infectious agents are absent, it is recommended that this product and all the samples be handled as though capable of transmitting infectious disease.

SAMPLE

• No haemolyzed fresh serum.

PROCEDURE

• Wavelength:	340 nm (334-365 nm)
• Pathlength:	1 cm
• Reading:	against air or Distil. water
• Temperature:	30-37°C
• Method:	kinetic
• Reaction:	5+5 minutes
• Linearity:	till 120 U/L
• Sample/Reagent:	1/15

Let reagent reaches the working temperature before using.

Pipette into a test tube or cuvette labelled:

S: Sample, ST: Calibrator/Standard/Control :

	ST	S
Working Reagent (*R1+*R2)	3000 µl	3000 µl
Calib./Standard/Control	200 µl	----
Sample	----	200 µl

Mix kindly and incubate for about 5 min. at the temperature of the test. Make the FIRST reading of Control (Astd1) and sample (As1). Repeat the SECOND readings after 5 min. for Control (Astd2) and sample (As2).

Determine the absorbance/5minutes for Control (ΔAstd/5min) and Sample (ΔAs/5min), as (FIRST reading – SECOND reading).

CALCULATION

Insert the means found in the following formula:

$$5'Nucleotidase (U/L) = \text{Control value} \times \frac{\Delta As/5min. \text{ Sample}}{\Delta Astd/5min. \text{ Control}}$$

REFERENCE VALUES

5'Nucleotidase	2,0 – 14,0 U/L	(30°C)
	3,0 – 20,0 U/L	(37°C)

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The NTP Reagent is linear up to 120 U/L.
For concentrations ≥ 120 U/L, dilute the sample 1:5 with saline sol., repeat the determ. and multiply the result $\times 5$.

Sensitivity: The minimum detectable is 1,5 U/L (37°C).

Within-run Precision:

	Mean (U/L) \pm 2s	CV %
Serum 1	14 \pm 1,2	1,8
Serum 2	54 \pm 4,1	1,2

Run-to-run (Day-to-day) Precision:

	Mean (U/L) \pm 2s	CV %
Serum 1	14 \pm 1,6	2,2
Serum 2	54 \pm 6,1	1,7

Interferences: See References point 2.

Correlation: A group of 20 serum from 3 to 82 U/L was assayed by this procedure and using a similar commercially available NTP Reagent. Comparison of the data gave following results:

Linear regression equation $y = 1,0123x - 0,22$
Correlation coefficient $r = 0,9915$ $n=20$

NOTE

1. Very deep attention must be given to interfering substances: endogenous ALP (Alk. Phosphatase) with activity greater than 300 U/L (30°C) may interfere with this assay; also ammonia, pyruvate and other keto-acids are removed during the first 5 minutes; certain drugs and other substances are able to influence levels of 5'Nucleotidase (see References 2.).
2. A proportional variation of reaction volumes do not modify the result.
3. If the activity is higher than 120 U/L dilute the sample 1:5 with saline solution and multiply the result $\times 5$.
4. We suggest not to mix Reagents from different Production lots.
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. Particular attention must be given to sample with very high conc. of AMMONIA ; it will consume some of the NADH, thereby reducing the maximum activity measurable.
7. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
8. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975)
3. Arkesteijn C.L.M., J. Clin. Chem. Clin.Biochem. 14, 155 (1976)

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