

# 5'-NUCLEOTIDASE ASSAY

Liquid Stable Assay

510(k) Exempt  
Health Canada Registered



## Excellent Performance

- Excellent precision with CV's of less than 5.0
- Extended linearity from 0 – 300 U/L

## Convenient

- Stable liquid stable format requires no reagent preparation
- Calibrator included with kit\*

## Excellent Reagent Stability

- Three month on-board stability
- 12 month kit stability

## Efficient

- Highly specific for hepatobiliary disease
- Rapid confirmation of hepatic origin of elevated Alkaline Phosphatase

## Flexibility

- High and low controls available
- Extended list of automated instrument and manual parameters

\*Packaged separately

**INNOVATIONS IN  
CLINICAL DIAGNOSTICS**



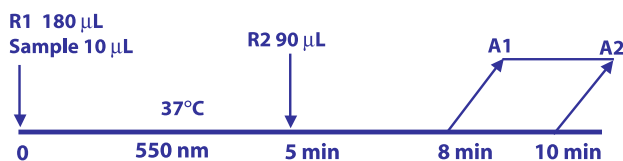
# 5'-NUCLEOTIDASE ASSAY

## SUMMARY OF PERFORMANCE

### Background

5'-Nucleotidase (5'-NT) is an enzyme which catalyzes the hydrolysis of nucleoside-5'-monophosphates such as AMP (adenosine-5'-phosphate) to nucleosides and inorganic phosphate. The enzyme is widely distributed in human and animal tissues. Increased enzyme activity is associated with certain forms of liver disease, such as intra- or extra-hepatic obstruction. Although both 5'-NT and Alkaline Phosphatase (ALP) behave similarly in hepatic disease 5'-NT is specific for hepatobiliary disease and unlike ALP, 5'-NT is not elevated during periods of increased osteoblastic activity, childhood and pregnancy. This increased specificity makes 5'-NT a useful marker in distinguishing between elevations in ALP due to hepatic and non-hepatic sources.

### Assay Method

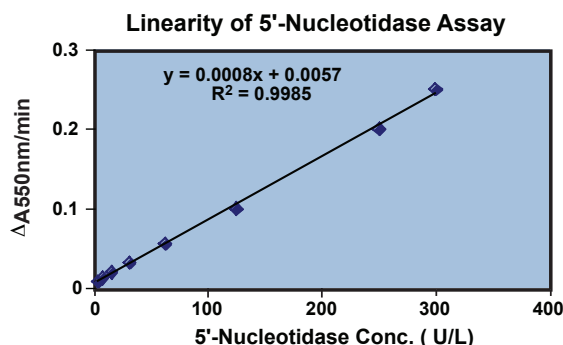


The 5'-NT assay is based on the enzymatic hydrolysis of 5'-monophosphate (5'-IMP) to form inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by xanthine oxidase (XOD). H<sub>2</sub>O<sub>2</sub> 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 kinetically.

### Performance

#### Linearity

The Diazyme 5'-Nucleotidase assay can be run via a k-factor or by calibrator. A single calibrator (which is included with the kit) is needed for running the assay in calibration mode. The k-factor for the assay was determined by using known concentrations of hydrogen peroxide titrated with the 5'-NT reagents. Based on the relation between the absorbance's and H<sub>2</sub>O<sub>2</sub> concentrations, the factor was calculated. Linearity was determined by running 2 replicates of a set of series diluted serum samples in one run. The assay is linear from 0 to 300 U/L.



### Precision

The precision of the Diazyme 5'-Nucleotidase assay was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study Intra-Assay Precision was determined by running fifteen (15) replicates of two (2) serum specimens with activity 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

### Interference

Assay is not affected by serum bilirubin up to 40 mg/dL, hemoglo-bin up to 500 mg/dL, triglycerides up to 1250 mg/dL, and ascorbic acid up to 20 mg/dL, alkaline phosphatase up to 1250 U/L.

## DIAZYME LABORATORIES

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