

Infinity™ Amylase Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	10 - 2000 U/L (0.167 - 33.4 µkat/L)
Specimen Type	:	Serum or Urine
Method	:	Kinetic
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		

INTENDED USE

This reagent is intended for the in vitro diagnostic use only, for the quantitative determination of α -Amylase (1,4- α -D-glucan glucanohydrolase EC3.2.1.1) in human serum or urine on both manual and automated systems.

CLINICAL SIGNIFICANCE^{1,2,3}

α -Amylase is derived mainly from the salivary glands and the exocrine pancreas. α -Amylase catalyses the hydrolysis of α -1 \rightarrow 4 glucosidic linkages of starch and other related polysaccharides to produce maltose and other oligosaccharides. The enzyme is a relatively small molecule which is rapidly cleared by the kidneys and excreted in the urine.

α -Amylase is most frequently measured in the diagnosis of acute pancreatitis when serum levels may be grossly elevated. In acute pancreatitis α -amylase starts to rise approximately 4 hours after the onset of pain, reaches a peak at 24 hours and remains elevated from 3-7 days.

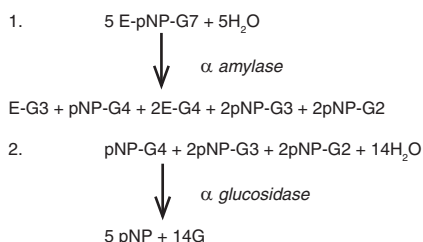
Hyperamylasaemia is also associated with other acute abdominal disorders, biliary tract disease, diabetic ketoacidosis, severe glomerular dysfunction, salivary gland disorders, ruptured ectopic pregnancy and macroamylasaemia.

METHODOLOGY

Several procedures are available for the assay of serum α -amylase activity. Amylolytic methods measure the disappearance of substrate and include the iodine-starch method. Saccharogenic methods measure the production of sugars such as maltose and glucose. Both these methods lack linearity, sensitivity and precision when compared to chromogenic methods which yield a colored product that can be measured spectrophotometrically.²

The Infinity Amylase Liquid Stable Reagent utilises Ethylidene-pNP-G7 (E-pNP-G7) as the substrate. The use of ethylidene prevents exo-enzymes from breaking down the substrate, so in the absence of α -amylase, no color change is observed. The substrate is also commonly referred to as EPS. Once the substrate has been cleaved by α -amylase, the smaller fragments produced can be acted upon by α -glucosidase, which causes the ultimate release of the chromophore.

The series of reactions involved in the assay system is as follows:



Where: G = Glucose
pNP = p-nitrophenol

The rate of formation of pNP is proportional to the α -amylase activity present in the sample and is measured by the rate of increase in absorbance at 405 nm (405-420nm).

REAGENT COMPOSITION

Active Ingredients	Concentration
E-pNP-G7	1.1 mmol/L
α -Glucosidase (microbial)	>3500 U/L
NaCl	51 mmol/L
Buffer	50 mmol/L
pH 7.0 \pm 0.1 at 20°C.	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Infinity Amylase Liquid Stable Reagent Material Data Safety Sheet.

REAGENT PREPARATION

Reagent is supplied ready to use.

STABILITY AND STORAGE

Prior to Use:

When stored at 2-8°C reagent is stable until the expiration date stated on the bottle and kit box label.

Once the Reagent is Opened:

When stored capped at 2-8°C, the reagent is stable until expiry.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent Absorbance >1.0 AU at 405nm (1cm); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.⁴

Urine: Random or timed collections are valid specimens.⁴

Storage: α -Amylase is exceptionally stable and serum samples may be stored for at least 7 days at room temperature and for at least 1 month at 4°C or -20°C.² Urine samples are stable for 7 days when stored at 4°C. If it is expected that there will be a delay in transporting the urine sample to the laboratory, the use of a chemical preservative such as Merthiolate (0.24 mmol/L) is recommended.⁵

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 405nm (405 - 420nm).
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	405 nm (405-420nm)
Secondary Wavelength	480-600nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1:40
eg : Sample Vol	7 µL
Reagent Vol	280 µL
Delay/Lag Time	60 seconds
Read Time	2 minutes
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 1.0 AU
Linearity	10 - 2000 U/L
(refer to linearity section)	(0.167 - 33.4 µkat/L)
Analytical Sensitivity	0.195 Δ mA/min per U/L
(405nm, 1cm lightpath)	(11.7 Δ mA/min per µkat/L)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = Δ Abs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000 \times 1.27}{\text{SV} \times \text{E} \times \text{P}}$$

where:

- TV = Total reaction volume in mL
- 1.27 = Method Conversion Factor (See note 2).
- SV = Sample Volume in mL
- E = millimolar extinction coefficient of pNP at 405nm = 10.13 (See note 1).
- P = Cuvette pathlength.

Example: Δ Abs/min = 0.02
 Factor = 5140
 Amylase = 0.02 x 5140 = 103 U/L

NOTES

- The millimolar extinction coefficient for pNP in this reagent system at 410nm = 9.80, 415nm = 9.17 and at 420nm = 8.30.
- With this factor the values obtained with the Infinity Amylase Liquid Stable Reagent at 405nm/37°C are comparable to those obtained with the previously available blocked PNP-G7 method.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- If the change in absorbance is greater than 0.39/min repeat the assay with less sample or dilute with saline. Remember to adjust the factor for the smaller sample volume or multiply the final result by the dilution factor.
- Valid results depend on accurately calibrated instrument, timing and temperature control.
- Unit conversion: U/L x 16.67 x 10⁻³ = μ kat/L.

CALIBRATION

Calibration is not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

- Studies to determine the level of interference from bilirubin (free & conjugated), haemoglobin and lipaemia were carried out using commercially available interference check products. The following results were obtained:

Haemoglobin: No interference from haemoglobin up to a level of 522 mg/dL.

Free bilirubin: No interference from free bilirubin up to a level of 265 μ mol/L (15.5mg/dL).

Conjugated bilirubin: No interference from conjugated bilirubin up to a level of 286 μ mol/L (16.7mg/dL).

Lipaemia: No interference from lipaemia, measured as an absorbance at 630nm, up to 1.045AU.

- Young DS[®] has published a comprehensive list of drugs and substances which may interfere with this assay.
- To avoid the possibility of contamination with α -amylase, please ensure that the reagent does not come in to contact with saliva and skin.

EXPECTED VALUES

Serum:* At 37°C 35 - 140 U/L (0.585 - 2.34 μ kat/L)
 At 30°C 27 - 108 U/L (0.451 - 1.80 μ kat/L)

Urine: 1 - 17 U/hour (0.017 - 0.284 μ kat/hour)⁷

*The quoted values were derived from a study of 59 normal samples and should serve as a guide only. The 30°C values were calculated as a guide only, using a temperature conversion factor of 0.77. We do not however recommend the routine use of temperature conversion factors. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁸

PERFORMANCE DATA

The following data was obtained using the Infinity Amylase Liquid Stable Reagent on a well maintained automated clinical chemistry analyser.

IMPRECISION:

Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.⁹

	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L / μ kat/L)	42 / 0.701	341 / 5.69
SD (U/L / μ kat/L)	2.2 / 0.037	3.2 / 0.053
CV (%)	5.3	0.9
Total: SD (U/L / μ kat/L)	3.4 / 0.057	8.9 / 0.149
CV (%)	8.1	2.6

METHOD COMPARISON:

Comparison studies were carried out using another similar commercially available α -amylase reagent. Serum and urine samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Serum:	
Number of sample pairs	60
Range of sample results	24 - 465 U/L (0.401 - 7.77 μ kat/L)
Mean of reference method results	67 U/L (1.12 μ kat/L)
Mean of Amylase Infinity results	69 U/L (1.15 μ kat/L)
Slope	0.9425
Intercept	1.8 U/L (0.030 μ kat/L)
Correlation coefficient	0.9970
Urine:	
Number of sample pairs	41
Range of sample results	5 - 595 U/L (0.084 - 9.94 μ kat/L)
Slope	1.187
Intercept	-7.6 U/L (0.127 μ kat/L)
Correlation coefficient	0.9953

LINEARITY:

When run as recommended, the assay is linear between 10- 2000 U/L (0.167 - 33.4 μ kat/L). Linearity on automated instruments will be dependent upon the ratio of sample volume to reagent volume used and the timing of measurements. The specific instrument application should be consulted.

ANALYTICAL SENSITIVITY:

When run as recommended the sensitivity of this assay is 0.195 Δ mA/min per U/L (11.7 Δ mA/min per μ kat/L).

REFERENCES

- JF Zilva and PR Pannall. "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke London 1979: Chapter XV: 341-2
- Foo YA and Brosalki SB. Ann Clin. Biochem 1986; 23:624-37
- Bais R. Am. Jnl of Clin. Path 1982; 78: 184-8
- Clinical Chemistry Infobase: A Scientific & Management Cyclopedia. Pesce-Kaplan Publishers 1996; 2619-2620.
- Shepherd MDS and Mazzachi RD. The Clin. Biochem 1983; 4: 61-7
- Young DS. Effects of Drugs on Clinical Laboratory Tests Third Edition 1990; 3: 34-6.
- Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994;2178.
- Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
- National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, 1984, NCCLS Publication EP5-T.



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Reorder Information

Catalogue No.	Configuration
TR25421	2 x 125 mL
1174-200H	4 x 50 mL (Hitachi)
TL25401	6 x 20 mL (ILab 600)
TR25456	2 x 28 mL
TY25401	4 x 53 mL

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