

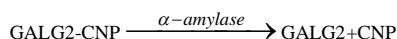
Reagent kit for the quantitative determination of alpha-amylase activity in serum and urine using GalG2-CNP substrate.

Measurements of amylase are used primarily in the diagnosis and treatment of the diseases of the pancreas.

Amylase is found primarily in the pancreas and salivary glands. When released in the digestive tract, the enzyme hydrolyzes starch. Amylase determinations are useful in the diagnosis of diseases of the pancreas and parotids. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis.

Principle

Alpha-amylase hydrolyzes 1,4-glucosidic linkages in starch and other polysaccharides to form short chain oligosaccharides. The substrate used in reagent is 2-chloro-4-nitrophenyl- α -galactosylmaltoside (GALG2-CNP). The rate at which p-nitrophenol is formed is directly proportional to the amylase activity in the sample. The resulting increase in absorbance can be measured spectrophotometrically at 405 nm.



Reference values

Serum: 15-100 U/l (0,25-1,67 μ kat/l)

Urine: ≤ 400 U/l ($\leq 6,67$ μ kat/l)

It is recommended that each laboratory should assign its own normal range.

Reagent

GALG2-CNP substrate	4.55 mmol/l
Buffers, pH=6.00	50 mmol/l
Calcium acetate	5 mmol/l
Sodium chloride	51.5 mmol/l
Preservatives	

Samples

Serum free of haemolysis, duodenum fluid and urine.

Urine: collect in clean and dry equipments and keep at 2-8°C until determination.

Chelating agents interfere with the reaction. Do not use citrate, oxalate or EDTA anti-coagulant. The reagent contains calcium, which can cause the precipitation of the fibrinogen from plasma.

Do not pipette by mouth and avoid contamination with skin! (Sweat and saliva contain alpha-amylase!)

PROCEDURE

Working reagent

The reagent is ready for use.

If the absorbance of working reagent is higher than 0.5 at 405 nm the reagent can not be used.

Assay conditions

Wavelength:	405 nm
Cuvette:	1 cm
Temperature:	37 °C
Method:	kinetic (increasing)

Pipette into cuvette

Working reagent	3 ml
Sample or control	50 μ l

Mix and incubate the reaction mixture at 37 °C for 1 minute.

Read absorbance values every 30 seconds after 1 minute for at least 2 minutes.

Determine the change of absorbance per minute ($\Delta A/\text{min}$).

Calibration

S1: Distilled water

S2: Roche C.F.A.S. liquid or

Randox Calibration Serum Level I or

Randox Calibration Serum Level II

Calibration frequency

Two point calibration is recommended:

- after reagent lot change,

- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance

C = Concentration

Calculation using factor

U/l = 4758 x $\Delta A/\text{min}$ (CFAS liquid); μ kat/l = 79,3 x $\Delta A/\text{min}$ (CFAS liquid)

$\Delta A/\text{min}$ = the change of absorbance per minute

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Hitachi 717 analyzer (37°C).

Linearity

The test is linear up to 3000 U/l (50 μ kat/l)

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 4.325 U/l (0,072 μ kat/l) alpha-amylase activity at 405 nm.

Precision

	Reproducibility		
	Average activity (U/l)	SD	CV%
Sample I.	187.8	2.4	1.28
Sample II.	475.1	8.7	1.83

Correlation

Comparative studies were done to compare our reagent with our Alpha-amylase EPS assay. The results from these studies are detailed below.

Correlation coefficient: r=0.989

Linear regression: y (U/l) = 1.021x + 4.729

(x = EPS reagent y = GALG2 reagent).

Specificity


Bilirubin 1026 μ mol/l (60 mg/dl), lipid 1000 mg/dl, glucose 111 mmol/l (2000mg/dl) and ascorbic acid 5.68 mmol/l (100mg/dl) don't interfere with the assay up to the given levels.


NOTE

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.


For in vitro diagnostic use only.

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

Street, H. V. and Close, J. R., Clin. Chem. Acta 1: 256 (1956)

Henry, R. J. and Chiamori, N. Clin. Chem. 6:434 (1960)

David, H., Clin. Chem. 28: 1485 (1985)

McCroskey, R., Chang, T., David, H. and Winn, E., Clin. Chem. 28: 1787, (1982)

Young, D. S., Effects of Drugs on Clinical Laboratory Tests, 3rd Edition AACC Press, 1990.