H-1117 Budapest,
Budafoki street 111-113
Tel.: +36-1-205-3617
Tel./Fax: +36-1-205-3616

ALPHA-AMYLASE (GALG2-CNP) CE

STABLE LIQUID REAGENT

Cat. No.:	45861	45863	45864
	120 ml	600 ml	20x20 ml

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.

$$\text{GALG2-CNP} \xrightarrow{\alpha-amylase} \text{GALG2+CNP}$$

Reference values
Serum:15-100 U/l (0,25-1,67 µkat/l)
Urine: ≤ 400 U/l (≤ 6,67 µkat/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 µl

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 (ΔA/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

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance
C = Concentration
Calculation using factor
U/l = 4758 x ΔA/min (CFAS liquid); µkat/l= 79,3 x ΔA/min (CFAS liquid)
ΔA/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 µkat/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 µkat/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 µmol/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

IVD For in vitro diagnostic use
Use by (last day of the month)
Temperature limitation
LOT Batch Code
REF 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.