

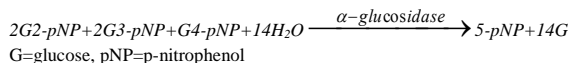
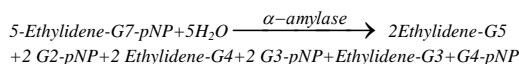
Cat. No.:	47461, 417463	47462, 417464	47463, 417465
	120 ml	600 ml	10x24 ml
	(1x100 ml+1x20ml)	(1x500 ml +1x100 ml)	(10x20 ml+ 10x4 ml)

Reagent kit for determination of the α -amylase activity in serum or urine based upon the IFCC EPS method.

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

The procedure utilizes a different auxiliary enzyme α -glucosidase, which cleaves all primary degradation products and leads to a 100% chromophore release from the substrate.



Reference values

Serum: 30-100 U/l (0,51-1,7 μ kat/l)

Urine: 50-500 U/l (0,85-6,67 μ kat/l)

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

Reagents

Reagent (R1)

HEPES buffer, pH=7.15	50 mmol/l
NaCl	70 mmol/l
MgCl ₂	10 mmol/l
α -glucosidase	≥ 4 kU/l

Reagent (R2)

4,6-Ethylidene-G7-pNP (EPS)	3 mmol/l
HEPES buffer, pH=7.15	50 mmol/l

Precaution

Discard cloudy reagent. Avoid contamination by using clean laboratory materials (pipettes, plastic vials...) for analyzers. These reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent. Do not use citrate, oxalate or EDTA anti-coagulant. Do not pipette by mouth and avoid contamination with skin! (Sweat and saliva contain alpha-amylase!)

Sample

Serum free of haemolysis and urine. Stability in serum: 1 month (2-8°C)

Stability

without opening:	till the expiry date indicated on the label
after opening:	30 days
calibration frequency:	7 days
onboard stability:	7-30 days

Stability data are valid only when using new system bottle!

PROCEDURE

Preparation and stability of working reagent

One-reagent procedure:
 Mix 5 volumes of R1 with 1 volume of R2.

Two-reagent procedure: The reagents are 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 (400-420) nm
Temperature:	37°C
Cuvette:	1 cm light path
Method:	kinetic (increasing)
Read against:	distilled water

If the absorbance of the reagent exceeds 0.5 at 405 nm, the reagent can not be used.

One-reagent procedure

Working reagent	600 μ l
Sample	8 μ l

Mix and after a 1-minute incubation, measure the change of absorbance per minute ($\Delta A/\text{min}$) for 3 minutes.

Two-reagent procedure

R1	500 μ l
Sample	8 μ l

Mix and wait 3 minutes.

R2	100 μ l
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Mix and after a 3-minute incubation, measure the change of absorbance per minute ($\Delta A/\text{min}$) for 3 minutes.

Calibration: (37°C, EPS method)

S1: Distilled water

S2: Diagnosticum DunaCal Cat. No.: Deal or 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

405 nm: activity (U/l) = $\Delta A/\text{min} \times 7280$ (CFAS liquid)

405 nm: activity (μ kat/l) = $\Delta A/\text{min} \times 121,3$ (CFAS liquid)

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. Recommended controls: Diagnosticum DunaCont N (Cat. No.: Dcon-N) and DunaCont P (Cat. No.: Dcon-P). DunaCont Urine two levels control (DCONU2) is recommended for urine analysis.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyser (37°C). Conversion factor: [U/l]=[μ kat/l] $\times 60$

Linearity

The method is linear in the range 10 – 1800 U/l (1,67 – 30 μ kat/l)

Limit of detection

The limit of detection is 0,69 U/l (0,012 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 7.28 U/l (0,12 μ kat/l) alpha-amylase activity at 405 nm.

Precision

n=20	serum		urine		
	Activity U/l	SD	CV%	Activity U/l	SD
206	2,49	1,21	21	1,2	5,74
457	5,22	1,14	78	4,2	5,37

Correlation

Comparative studies were done to compare our reagent with another commercial alpha-amylase (EPS) assay.

The results from these studies are detailed below.

Correlation coefficient: $r = 0,9969$

Linear regression: y (U/l) = $0,991x - 5,645$

(x= other commercial reagent, y= own reagent).

Specificity










Bilirubin 1026 μ mol/l (60 mg/dl), lipid 1000 mg/dl, glucose 111 mmol/l (2000 mg/dl) and ascorbic acid 5.68 mmol/l (100 mg/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 can be used on the labels

	In vitro diagnostic device		Batch code
	Manufacturer		Catalogue number
	CE-marking		This way up
	Temperature limitations		Biological risk
	Use by (year/month)		

Bibliography

A. Kurrie-Weittenhiller, W.Hölzel, D.Engel, J.Finke, G. Klein: Clin.Chem.42, 598(1996)
 Szabó A.: Klinikai laboratóriumi vizsgálatok és paraméterek (2010) (ISBN 978-963-9879-75-1)

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