ALFA AMILASE G7

ALPHA AMYLASE G7

Anvisa 80115310093

ORDER INFORMATION

Cat. No.	Kit size
2080075K	R1: 3 x 20 mL + R2: 1 x 15 mL
2080075M	R1: 3 x 20 mL + R2: 1 x 15 mL
2080112.4R	R1: 4 x 21,3 mL + R2: 4 x 6,8 mL
2080050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Alpha-amylases in serum, plasma or urine on photometric systems.

SUMMARY [1,2]

α-Amylases are hydrolytic enzymes which break down starch into maltose. In the human body, α-amylases originate from various organs: pancreatic amylase is produced by the pancreas and released into the intestinal tract; salivary amylase is synthesized in the salivary glands and secreted into saliva. Amylases present in blood are eliminated through the kidney and excreted into urine. Therefore, elevation of amylase activity in serum is reflected in a rise of urinary amylase activity. Measurement of α-amylases in serum and urine is mainly used to diagnose pancreatic disorders as well as for detecting the development of complications. In acute pancreatitis the blood amylase activity increases within a few hours after onset of abdominal pain, peaks after approx. 12 h and returns to values within the reference range at the latest after 5 days. The specificity of α-amylases for pancreatic disorders is not very high as elevated levels are measured also in various non-pancreatic diseases, e.g. parotitis and renal insufficiency. Therefore, for confirmation of an acute pancreatitis, lipase should be measured in addition.

METHOD

Enzymatic photometric test, in which the substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α-D-maltoheptaoside (EPS-G7) is cleaved by α-Amylases into various fragments. These are further hydrolyzed in a second step by α-Glucosidase producing glucose and p-nitrophenol. The increase in absorbance represents the total (pancreatic and salivary) amylase activity in the sample. [3,4]

PRINCIPLE

$$5\ \text{EPS-G7} + 5\ H_2O \xrightarrow{\alpha\text{-Amylase}} \begin{matrix} 2\ \text{Ethylidene-G5} + 2\ \text{G2PNP} \\ +\ 2\ \text{Ethylidene-G4} + 2\ \text{G3PNP} \\ +\ \text{Ethylidene-G3} + \text{G4PNP} \end{matrix}$$

$$2\ \text{G2PNP} + 2\ \text{G3PNP} + \text{G4PNP} + 14\ H_2O \xrightarrow{\alpha\text{-Glucosidase}} 5\ \text{PNP} + 14\ \text{G}$$

(PNP = p-Nitrophenol, G =Glucose)

REAGENTS

Components and Concentrations

R1	Good's Buffer	pH 7.15	0.1 mol/L
	NaCl		62.5 mmol/L
	$MgCl_2$		12.5 mmol/L
	α-Glycosidase		≥ 2 KU/L
R2	Good's Buffer	pH 7.15	0.1 mol/L
	EPS-G7 (4,6-ethylidene-(G7)-p-Nitrophenyl-(G1)-α-D-maltoheptaside)		8.5 mmol/L

STORAGE AND STABILITY

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C, protected from light and if contamination is avoided. Do not freeze the reagents!

WARNINGS AND PRECAUTIONS

1. Saliva and skin contain alpha amylase, so never pipette reagents by mouth and avoid contact of reagents with the skin.
2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1 contains animal and biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [5].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only.

WASTE MANAGEMENT

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

Starting with Substrate

The reagents are ready to use.

Starting with Sample

Mix 4 parts of R1 with 1 part of R2

(e.g. 20 mL R1 + 5 mL R2) = monoreagent

Stability: 4 weeks at 15 - 25 ºC
6 months at 2 - 8 ºC

Protect the monoreagent from light!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Serum, heparin plasma or urine

Stability [6]:

Serum or plasma:	7 days	at	20 - 25 °C
	7 days	at	4 - 8 °C
	1 year	at	-20 °C
Urine:	2 days	at	20 - 25 °C
	10 days	at	4 - 8 °C
	3 weeks	at	-20 °C

Only freeze once!
Discard contaminated specimens!

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

Wavelength	Hg 405 nm
Optical path	1 cm
Temperature	37 °C
Measurement	Against reagent blank

Starting with Substrate

	Serum or plasma		Urine	
	Blank	**Sample**	**Blank**	**Sample**
Sample or calibrator	-	20 µL	-	10 µL
Distilled water	20 µL	-	10 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL	1000 µL
Mix, incubate for approximately 1 min, then add:				
Reagent 2	250 µL	250 µL	250 µL	250 µL
Mix, read the absorbance (A1) after 2 min and start the stopwatch. Read the absorbance again after 1, 2 and 3 min.				

Starting with Sample

	Serum or plasma		Urine	
	Blank	**Sample**	**Blank**	**Sample**
Sample or calibrator	-	20 µL	-	10 µL
Distilled water	20 µL	-	10 µL	-
Monoreagent	1000 µL	1000 µL	1000 µL	1000 µL
Mix, read the absorbance (A1) after 2 min and start the stopwatch. Read the absorbance (A2) again after 1, 2 and 3 min.				

CALCULATION

With factor

From the absorbance readings, calculate the ΔA/min and multiply by the corresponding factor from the table below:

ΔA/min x Factor = Amylase Activity [U/L]

	Starting with Substrate	Starting with Sample
Serum or plasma	5670	4554
Urine	11250	9018

With calibrator

$$\text{Alpha-Amylase [U/L]} = \frac{\Delta A/\text{min}_{\text{Sample}}}{\Delta A/\text{min}_{\text{Cal.}}} \times \text{Conc. Cal. [U/L]}$$

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. This method has been standardized against the original IFCC formulation. Use Kovalent Topkon N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

PERFORMANCE CHARACTERISTICS

Measuring range

In automated systems the test is suitable for the determination of α-Amylase activity up to 2000 U/L.
In the case of manual procedure, the test is suitable for determination of α-Amylase activity, which corresponds to a maximum of ΔA/min = 0.35.
If these values are exceeded, the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and the results multiplied by 10.

Specificity / Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin (conjugated and unconjugated) up to 40 mg/dL, hemoglobin up to 550 mg/dL, and lipemia up to 1000 mg/dL of triglycerides. For more information on interfering substances, see Young DS [7].

Sensitivity / Limit of Detection

The lowest detection limit is 3 U/L.

Precision

Within run n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	184	2.00	1.08
Sample 2	398	2.67	0.67
Sample 3	841	4.96	0.59

Total Precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	180	1.82	1.01
Sample 2	383	3.74	0.97
Sample 3	817	7.48	0.92

Method comparison

Method comparison between Kovalent Alfa Amilase G7 (y) and a routine recommended method (x) using 51 samples demonstrated the following results: y = 0.964 x – 2.455 U/L; r = 0.998

Method comparison between Kovalent Alfa Amilase G7 (y) and a available commercial test (x) using 51 samples demonstrated the following results: y = 1.031 x – 3.613 U/l; r = 0.994

REFERENCE VALUES [8]

	Women	Men
Serum/plasma	< 100 U/L (< 1.67 µkat/L)	< 100 U/L (< 1.67 µkat/L)
Urine	< 447 U/L (< 7.45 µkat/L)	< 491 U/L (< 8.18 µkat/L)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine its own reference ranges if necessary.

LITERATURE

1. Lorentz K. alfa Amylase. In: Thomas L, editor. Clinical laboratory diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998.p.46-51.
2. Moss DW, Henderson AR. Digestive enzymes of pancreatic origin. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company;1999.p.689-98.
3. Kruse-Jarres JD, Kaiser C, Hafkenscheid JC, Hohenwallner W, Stein W., Bohner J et al. Evaluation of a new alpha amylase assay using 4,6-ethylidene-(G7)-1-4-nitrophenyl-(G1)-alpha-D-maltoheptaoside as substrate. J Clin Chem Biochem 1989;27:103-13.
4. Schumann G, Bonora R, Ceriotti F Férar G et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37°C. Clin Chem Lab Med 2006;44(9):1146-1155.
5. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
6. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 50-1.
7. Young DS. Effects of Drugs on Clinical Laboratory Testes. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
8. Junge W, Wortmann W, Wilke B, Waldenstroem J et al. Development and evaluation of assays for determination of total and pancreatic amylase at 37 °C according to the principle recommended by the IFCC. Clin Biochem 2001;34:607-15.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	*In vitro* diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:
Kovalent do Brasil Ltda.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414 - Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310093	R1: 2 x 50 mL + R2: 2 x 12,5 mL
80115310093	R1: 3 x 26,67 mL + R2: 1 x 20 mL
80115310093	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310093	R1: 3 x 40 mL + R2: 3 x 10 mL
80115310093	R1: 4 x 40 mL + R2: 4 x 10 mL

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Expiration date and Lot no.: See label