α - AMYLASE - EPS

Enzymatic - Colorimetric Method
E.C.3.2.1.1.

Cat.No. 101-0157	*Size 20 x 3 ml*
Cat.No. 101-0150	*Size 10 x 10 ml*
Cat.No. 101-0196	*Size 4 x 50 ml*

PRINCIPLE:

As substrate in this method ethylidene-p-nitrophenyl-α,D-maltoheptaoside (ethylidene-G7PNP) is used. By effecting of α-amylase (cleavage of internal bonds) and α-glucosidase, p-nitrophenol is liberated. The increase of absorbance per minute at 405 nm is directly proportional to the α-amylase activity.

$$5\text{ethylidene} - G_7PNP + 5H_2O \xrightarrow{\alpha-\text{amylase}} 2\text{ethylidene} - G_5 + 2G_2PNP + 2\text{ethylidene} - G_4 + 2G_3PNP + \text{ethylidene} - G_3 + G_4PNP$$

$$2G_2PNP + 2G_3PNP + 10H_2O \xrightarrow{\alpha-\text{glucosidase}} 10G + 4p - \text{nitrophenol}$$

(PNP = p - nitrophenol; G = glucose)

SAMPLE:

Serum, heparinized plasma, urine. Stability 7 days at +15 °C to +25°C, 2 months at +2 °C to +8 °C.

REAGENTS:

1. Buffer	
Hepes buffer, pH 7.1	100 mmol/L
Sodium chloride	50 mmol/L
Magnesium chloride	10 mmol/L
2. Substrate/enzyme	
4,6 - ethylidene - G_7PNP	3 mmol/L
α-Glucosidase	> 23 U/mL

Store the reagents at +2 °C to +8 °C.

PREPARATION OF REAGENTS:

Reconstitute the contents of one vial of substrate (vial 2) with the corresponding volume of buffer (vial 1), by shaking gently. Working reagent is stable for 28 days at +2°C to +8°C or 5 days at +15°C to +25°C.

PROCEDURE:

Method:	Kinetic (increasing)
Wavelength:	405 nm (400 - 420 nm)
Cuvette:	1 cm light path
Temperature:	25 °C, 30 °C, 37 °C
Zero:	air

Pipette into cuvette:				
	+25 °C / +30 °C		+37 °C	
	Serum	Urine	Serum	Urine
Working Reagent	1000 µl	1000 µl	1000 µl	1000 µl
Sample	50 µl	25 µl	25 µl	10 µl
Mix and incubate 3 minutes at +25 °C or 30 °C or 2 minutes at 37°C. Then read absorbance and at the same time start stop-watch. Read absorbance again after exactly 1, 2 and 3 minutes. Calculate $\Delta A_{405\ nm}$/min.				

CALCULATION:

Calculate the activity of α-amylase in the sample (in U/L) as follows:
ΔA/min x Factor = U/L α-amylase

	Factor		
Temperature	25 °C	30 °C	37 °C
Serum, plasma	6630	6630	12943
Urine	12943	12943	31883

To convert the results to other methods with blocked substrat G_7pNP divided by the factor 2.91.

EXPECTED VALUES:

	25 °C	30 °C	37 °C	UNIT
Serum, Plasma	≤ 120 ≤ 2000	≤ 160 ≤ 2267	≤ 220 ≤ 3367	U/L nkat/L
Urine	≤ 600 ≤ 10000	≤ 800 ≤ 13336	≤ 1000 ≤ 16670	U/L nkat/L
24 h urine	≤ 450 ≤ 7500	≤ 650 ≤ 10836	≤ 900 ≤ 15000	U/L nkat/L

U/L x 16.67 = nkat/L

LINEARITY:

at 25 °C to 30 °C: up to 1990 U/L (33170 nkat/L)
at 37 °C: up to 3883 U/L (64730 nkat/L)

NOTE:

1. Interfering substances: the presence of citrate, oxalate and EDTA interfere because thay bind the magnesium ion which is important for amylase activity. Only heparin as anticoagulant can be used.
2. Lipemia hinders amylase determination.
3. If ΔA/min > 0.300 dilute the sample 1:5 with saline solution. The value has to be multiplied by 5.
4. If wavelength is not exactly 405 nm or thermostation is not fine the factor must be determined using control sera whose α-amylase value is known.
5. Saliva and sweat contain α-amylase. To reduce the possibility of contamination do not pipette by mouth and avoid contact of the sample, reagent and pipette tips with the skin.
6. Any slight changes in the yellow coloration of the working reagent do not interfere with the performance of the test.

QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

REFERENCE:

1. Rauscher E., et al., J. Clin. Chem. Clin. Biochem. 23, 588, 1985