POINTE SCIENTIFIC, INC.
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Amylase (EPS-G7) Liquid Reagent Set

Intended Use
For the quantitative kinetic determination of α-amylase activity in human serum. For *in vitro* diagnostic use only.

Clinical Significance
The determination of amylase activity in serum is most commonly performed for the diagnosis and treatment of diseases of the pancreas.

Method History
Amylase was first measured quantitatively by an iodometric method introduced by Wohlegemuth in 1908.[1] Somogyi introduced a procedure in 1938 that standardized the amounts of starch and iodine.[2] His work became the basis for the widely used Amyloclastic and Saccharogenic methods introduced in 1956[3] and 1960[4], respectively. Rinderknecht et al introduced a dye-coupled starch in 1967[5] that was relatively simple to perform. However, the procedure used an insoluble substrate, lacked linearity, and still required centrifugation or filtration.

Turbidimetric procedures have been introduced[6] but they are now rarely used. Several enzymatic procedures have been suggested[7,8] including one that used the defined substrate maltotetraose.[9] These methods represented significant improvement in amylase measurement, but were still subject to relatively long preincubation times, possible endogenous glucose interference, and a series of other potential interferences with the formation of NADH.[10]

Wallenfels et al[11] introduced ρ-nitrophenylglycosides as defined substrates for α-amylase determination in a procedure that eliminated interference from endogenous glucose and pyruvate. The present method is based on the use of 4,6-ethylidene (G7)- ρ -nitrophenyl(G1)- α,D-maltoheptaoside (EPS-G7) as the substrate. The addition of the ethylidene group to this ρ-nitrophenyl-glycoside improves the stability of the reagent by protecting the substrate from residual amylase activity. The rate of increase in absorbance is measured spectrophotometrically at 415nm, and is used to determine amylase activity.

Principle
$$5EPS\text{-}G7 + 5H_2O \xrightarrow{\alpha\text{-Amylase}} 2ET\text{-}G5 + 2G2PNP + 2ETG4 + 2G3PNP + ETG3 + G4PNP$$

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

α-Amylase hydrolyzes the substrate (EPS-G7) to produce oligosaccharides. The α-glucosidase hydrolizes the oligosaccharides liberating ρ-nitrophenol. The rate of increase in absorbance is measured at 415nm and is proportional to the α-amylase activity in the sample.

Reagents
When combined the reagent contains the following components:

Buffer (pH 7.2±0.1)	50 mM
NaCl	>50 mM
$MgCl_2$	10mM
α-Glucosidase	>2KU/L
EPS-G7	>1.0mM

Precautions
1. This reagent kit is intended for in vitro diagnostic use only.
2. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2) [12]

Reagent Preparation
The reagent is provided as a two-part, ready-to-use liquid.

Reagent Storage
1. Store reagents at 2-8°C.
2. The reagents are stable until the expiration date if stored as directed.

Reagent Deterioration
Do not use if:
1. The reagent fails to meet stated parameters of performance.
2. The reagents are turbid or display other evidence of bacterial contamination.

Specimen Collection and Handling
1. Unhemolyzed serum is the specimen of choice. Specimens should be collected as per NCCLS document H4-A3.[13]
2. Anticoagulants, such as Citrate and EDTA, bind calcium that is needed for amylase activity. Plasma with these anticoagulants should not be used.
3. Amylase in serum is reported stable for one week at room temperature (18-25°C.) and for two months when stored refrigerated at 2-8°C.[14]
4. Specimen collection should be carried out in accordance with NCCLS M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences
1. A number of drugs and substances affect the determination of amylase.[15,16] Young et al have published a comprehensive list of such substances.[17]
2. The presence of macroamylase in the specimen can cause a measured hyperamylasemia that could lead to a false diagnosis of acute pancreatitis. However, no clinical symptoms are usually associated with macroamylasemia.[18]
3. Bilirubin (20mg/dl) and hemoglobin (500mg/dl) have each been found to have a negligible effect on this procedure.
4. Lipemic samples up to 1000 mg/dl have been reported to have no effect on serum amylase determinations.[19]

Materials Provided
Amylase (EPS-G7) reagents

Materials required but not Provided
1. Chemistry Controls
2. Beckman Coulter AU™ analyzer
3. Application and instrument manuals

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: **#** TEST NAME: **Amylase** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **6** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **30** DIL. VOL.: **0** L **0.000** H **2.500**
REAGENT OD LIMIT:
WAVELENGTH: PRI. **410** ∇ SEC. **700** ∇ FIRST L: **-0.1000** FIRST H: **1.500**
METHOD: **RATE** ∇ LAST L: **-0.1000** LAST H: **1.500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **17** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: **25** % A: **1.000** B: **0.000**
NO LAG TIME: **NO** ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: # ∇ LEVEL L: **#** LEVEL H: **#**

NORMAL RANGES:		AGE L		AGE H			
	SEX	YEAR	MONTH	YEAR	MONTH	L	H
○	1. # ∇	#	#	#	#	#	#
○	2. # ∇	#	#	#	#	#	#
○	3. # ∇	#	#	#	#	#	#
○	4. # ∇	#	#	#	#	#	#

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○	5.	#	∇	#	#	#	#	#	#
○	6.	#	∇	#	#	#	#	#	#
	7.	NONE SELECTED						#	#
	8.	OUT OF RANGE			L	H		#	#

PANIC VALUE: # # UNIT: **U/L** DECIMAL PLACES:**0**

CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **MB** ∇ FORMULA: **Y+AX+B** ∇ COUNTS: **2** PROCESS: ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#		
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: **3647** CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with you laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Limitations

1. Samples exceeding the linearity limit (2,000 U/L) should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. The presence of macroamylase in the specimen can cause a measured hyperamylasemia that could lead to a false diagnosis of acute pancreatitis. However, no clinical symptoms are usually associated with macroamylasemia.[18]

Calibration

The procedure is standardized by means of the millimolar absorptivity of 2-chloro-ρ-nitrophenol which is 10.6 at 415nm under the conditions described.

Calculation

$$\frac{\Delta Abs/min \times TV \times 1000}{MMA \times SV \times LP} = U/L\ \alpha\text{-amylase in sample}$$

$$\frac{\Delta Abs/min \times 1.025 \times 1000}{10.6 \times 0.025 \times 1.0} = \Delta Abs/min. \times 3868 = U/L\ \alpha\text{-amylase}$$

Example: If ΔAbs/min = 0.03, then 0.03 x 3868 = 116 U/L

SI Units

To convert to SI units (nKat/L) multiply the U/L value by 16.67.

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal amylase values. These controls should be run at least with every working shift in which amylase assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Expected Values

Serum: 25-125 U/L for a similar kinetic method.[20] The expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance

1. Linearity: 1-2,000 U/L
2. Sensitivity: The sensitivity for the amylase reagent was investigated by reading the change in absorbance per minute at 410 / 520 nm for a saline sample, and a serum with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the amylase reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L amylase activity gives a ΔAbs/min. of 0.0002.
3. Comparison: A comparison study performed between the Beckman Coulter AU™400 and Hitachi 717 using this method resulted in a correlation coefficient of 0.999 and the linear regression equation was y=1.096x - 5.7.
4. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

Within Day (N=20)			Day To Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
72	0.9	1.3	278	6.6	2.4
591	7.4	1.3	613	7.4	1.2
1136	17.1	1.5			

Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP6 using a Beckman AU™400 analyzer [21]

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