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IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 1023Z

Enzyme Immunoassay

Amylase

Cat # 1023Z

For *in vitro* Research Use Only

NAME AND INTENDED USE

The Diagnostic Automation . Pancreatic Amylase Quantitative is a solid phase enzyme linked immunosorbent assay. This test provides quantitative measurement of human pancreatic amylase (h-p-Amylase) in serum and urine to aid in management of patients with pancreatic diseases. (*For Professional Use Only*)

SUMMARY AND EXPLANATION OF TEST

Human alpha-amylase (EC 3.2.1.1) consists of two major isoenzymes, pancreatic and salivary. Pancreatic amylase is synthesized only in pancreatic tissue, whereas the salivary-type amylase has been found in salivary glands, pulmonary, female genital, hepatic and various malignant tissues[1]. The alpha-amylase present in blood and urine of normal individuals is predominantly of pancreatic and salivary origin. The pancreatic enzyme appears to account for less than 50% of serum amylase[2].

Increase serum alpha-amylase in acute pancreatitis presumably results from escape of enzyme into the interstitial tissue and peritoneal cavity, with increased absorption through the lymphotics and veins. Amylase determinations are widely used to diagnosis and evaluation of pancreatic diseases[3, 4]. However determinations of total amylase give misleading information [5, 6]. Many of the methods available for separating amylase isoenzymes are laborious and time-consuming[2]. A method has become available in which monoclonal antibody is used to inhibit the salivary isoenzyme with negligible cross-reactive with pancreatic isoenzyme[7].

DAI h-p-Amylase Quantitative provides a direct enzyme immunoassay in which pancreatic amylase is specifically recognized both by the solid phase anti-bodies and conjugate antibodies. This eliminates the interference of the other isoamylase and the problems derived from non-standardized substrates preparation and conditions that employed in most of the enzymatic assays.

PRINCIPLE OF THE ASSAY

The DAI h-p-Amylase Quantitative is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with anti-h-p-amylase antibodies. The samples are incubated in the wells with enzyme conjugate which is a mixture of anti-h-p-Amylase antibodies chemically conjugated with horseradish peroxidase. Unbound conjugate is washed off with water. The enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the h-p-Amylase present in the samples. Upon addition of the Substrate and Chromogen, the intensity of color developed is proportional to the concentration of h-p-Amylase in the samples.

WARNINGS AND PRECAUTIONS

1. DAI h-p-amylase Quantitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

STORAGE AND STABILITY

1. Store the kit at 2-8°C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The unopened reagents are stable until expiration of the kit.
3. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose test reagents to strong light during storage or usage.

MATERIALS PROVIDED

1. Micro-wells strips (96 wells): Anti-h-p-amylase antibodies coated wells, 96 wells.
2. Sample Diluent (11 mL): 1 bottle
3. Enzyme conjugate (11 mL): Anti-h-p-amylase antibodies conjugated to horseradish peroxidase.
4. Reference Standard Set (0.3 mL each vial)
   The concentrations are 0, 10, 50, 150, 300 and 600 U/L.
5. Solution A (11 mL): Buffer solution containing hydrogen peroxide.
6. Solution B (11 mL): Tetramethyl Benzidine solution.
7. Stop Solution: 2 N HCl.
8. Well holder: For securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader
2. Pipetor with tips for 10 uL, 50 uL & 100 uL

SAMPLE COLLECTION AND HANDLING

Collect blood aseptically by venipuncture, allow to clot. Separate the serum by centrifugation at room temperature, and store in sterile tubes. If sera cannot be assayed immediately, they can be stored at 2-8°C for a month or frozen at -20°C for up to 6 months. Avoid repeated freezing and thawing of serum specimens.

PREPARATION FOR ASSAY

1. Before beginning the test, bring all samples and reagents to room temperature (20-25°C) and shake gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent results.
3. Use new disposable tips for each sample.

ASSAY PROCUDURE

1. Secure the desired number of coated wells in holder. Mark data sheet with sample identification.
2. Dispense 10 uL of Standards and patient samples into appropriate wells.
3. Dispense 100 uL of enzyme conjugate into each well.
4. Incubate for 60 minutes at room temperature.
5. Rinse the wells 5 times with running tap water.
6. Dispense 100 uL of Solution A and 100 uL of Solution B into each well.
7. Incubate for 30 minutes at room temperature.
8. Stop reaction by adding 50 uL of 2 N HCl solutions to each well and read OD at 450 nm with microwell reader.

PROCEDURAL NOTE

1. It is very important to wash the microwells thoroughly and remove the last droplets of water to achieve the best results.
2. Pipet all reagents and samples into the bottom of well. Avoid scratching the well. Vortex-mixing or shaking of wells is not required.
3. Absorbance is the function of the time and temperature of incubations. It is recommended to have all reagents and samples caps removed. All needed wells secured in holder and assigned. This will ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason, the size of the assay run should be limited. It is suggested to run no more than 20 patients with a set of Reference Standards in duplicate.
5. If in an assay, a serum specimen has been found to contain greater than 1,000 U/L of h-p-amylase, the sample must be further diluted with sample diluent and re-assayed as described in Assay Procedure.

CALCULATION OF RESULTS

Any microwell reader capable of determining absorbance at 450 nm may be used. The h-p-amylase value of patient is obtained as follows:

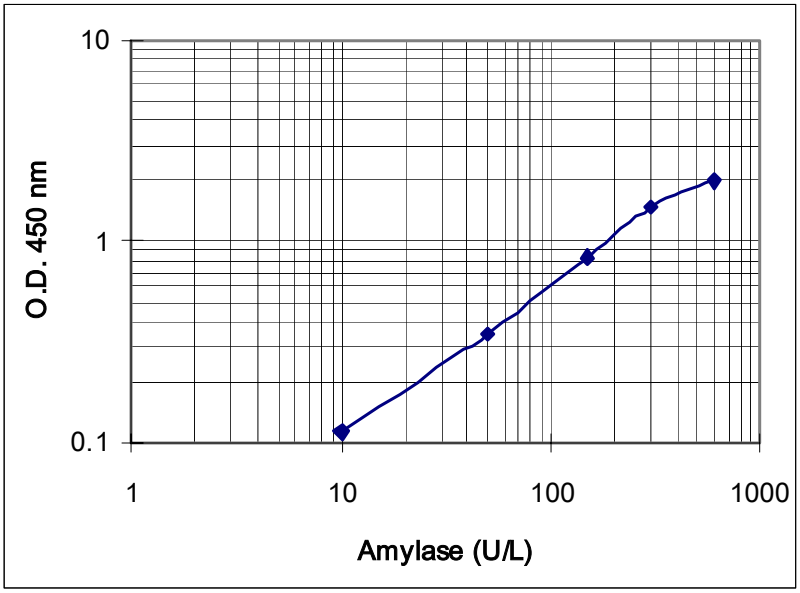
1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on graph paper.

2. Obtain the h-p-amylase values of samples by reference to the standard curve. The table and the figure are the example. The data is for demonstration purpose only and must not be used in place of data generated for each assay.

Well No.	Descrption (U/L)	Absorbnce (450 nm)	Value from Std. Curve
A1	0 U/L	0.000	
B1		0.000	
A2	10 U/L	0.116	
B2		0.112	
A3	50 U/L	0.276	
B3		0.262	
A4	150 U/L	0.828	
B4		0.848	
A5	300 U/L	1.469	
B5		1.499	
A6	600 U/L	1.971	
B6		2.035	
A7	Patient A	0.602	200
B7	(serum)	0.591	

LIMITATIONS

1. For diagnostic purpose, the h-p-amylase values should be used as an adjunct to other data available to the physician.
2. The DAI h-p-amylase Quantitative kit has been designed to avoid “hook effect” at the concentration of h-p-amylase up to 10,000 U/L
3. Sample with h-p-amylase level above 1,000 U/L should be diluted to obtain an accurate value.



EXPECTED VALUE

1. It is recommended that each laboratory determine its own normal and abnormal range.
2. For reference purpose, a reported of serum h-p-amylase is listed as following (7)

N = 400	
median	30 U/L
2.5 percentile	13 U/L
97.5 percentile	64 U/L

QUALITY CONTROL

Good laboratory practice includes the use of control specimens to ensure that all reagents and protocols are performing properly. DAI h-p-amylase assay do not include serum controls. Controls at different levels to monitor assay performance should be established.

The controls should be treated as unknown. Values obtained should be in agreement with the assigned values of the controls. Control should not contain sodium azide as preservatives.

PERFORMANCE CHARACTERISTICS

Accuracy
Recovery studies were performed by mixing an aliquot of pooled serum and h-p-amylase standard. The h-p-amylase values were measured and percentage of recovery determined.

Initial Values (U/L)	Concentration Spiked (U/L)	Expected Values (U/L)	Observed Values (U/L)	Recovery (%)
27	80	54	56	103
27	250	138	135	98
80	230	155	145	94
90	250	170	170	100
230	540	385	350	91
240	520	380	340	89

Precision
Intra-assay and Inter-assay coefficient of variation were evaluated at three different pooled serum samples:

Inter-Assay	Pool A	Pool B	Pool C
N	10	10	10
Mean (U/L)	123	338	705
S.D. (U/L)	12.1	30.1	39.5
C.V. (%)	9.8	8.9	5.6

Intra-Assay	Pool A	Pool B	Pool C
N	10	10	10
Mean (U/L)	120	330	700
S.D. (U/L)	8.7	22.1	34.3
C.V. (%)	7.3	6.7	4.9

Specificity
The human salivary amylase concentration from 10 U/L to 100,000 U/L were tested for the interference and cross-reactivity of this assay. There is no cross-reactivity observed.

The specificity of the h-p-amylase assay was also determined by studying the interference of transferring, gamma globulins, bilirubin, triglycerides and hemoglobin. No significant interference was observed at levels encountered in routine testing

Sensitivity
A linearity study was performed to assess the sensitivity of the assay.

Initial (U/L)	Dilution Fold	Expected (U/L)	Observed (U/L)	Recovery (%)
50	1:2	50	48	96
	1:4	25	25	100
	1:8	2.5	13	104
	1:16	6.25	6	94

MINIMAL DETECTABLE CONCENTRATION

The minimal detectable concentration of h-p-amylase is estimated to be 4.6 U/L. The minimum detectable concentration is defines as that concentration of h-p-amylase which corresponds to this absorbance value that is two standard deviations greater than the mean absorbances value of twenty replicate determination of the zero diluent.

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Date Adopted	Reference No.
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