UMA CO., LTD.

2-19-6 Yokosuka

Matsudo, Chiba, Japan

MEASURE AMYG7

Reagent for determination of Amylase activity

G7CNP IFCC Method

2 - 8°C	IVD *In vitro* Diagnostics	QUALITY MANAGEMENT SYSTEM (BY TUV)
DO NOT freeze	18 months/block from light	ISO 13485:2016

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Amylase (AMY) activity in serum, plasma and urine.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.

b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.

c. For guaranteed results, usage of this product must comply with the instruction in this manual.

d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

Amylase, any member of a class of enzymes that catalyze the hydrolysis (splitting of a compound by addition of a water molecule) of starch into smaller carbohydrate molecules such as maltose (a molecule composed of two glucose molecules). Three categories of amylases, denoted alpha, beta, and gamma, differ in the way they attack the bonds of the starch molecules.

Alpha-amylase is widespread among living organisms. In the digestive systems of humans and many other mammals, an alpha-amylase called ptyalin is produced by the salivary glands, whereas pancreatic amylase is secreted by the pancreas into the small intestine. The optimum pH of alpha-amylase is 6.7 - 7.0.

Ptyalin is mixed with food in the mouth, where it acts upon starches. Although the food remains in the mouth for only a short time, the action of ptyalin continues for up to several hours in the stomach - until the food is mixed with the stomach secretions, the high acidity of which inactivates ptyalin. Ptyalin's digestive action depends upon how much acid is in the stomach, how rapidly the stomach contents empty, and how thoroughly the food has mixed with the acid. Under optimal conditions as much as 30 to 40 percent of ingested starches can be broken down to maltose by ptyalin during digestion in the stomach.

When food passes to the small intestine, the remainder of the starch molecules are catalyzed mainly to maltose by pancreatic amylase. This step in starch digestion occurs in the first section of the small intestine (the duodenum), the region into which the pancreatic juices empty. The by-products of amylase hydrolysis are ultimately broken down by other enzymes into molecules of glucose, which are rapidly absorbed through the intestinal wall

Beta-amylases are present in yeasts, molds, bacteria, and plants, particularly in the seeds. They are the principal components of a mixture called diastase that is used in the removal of starchy sizing agents from textiles and in the conversion of cereal grains to fermentable sugars. Beta-amylase has an optimum pH of 4.0 - 5.0.

Gamma-amylases are known for their efficiency in cleaving certain types of glycosidic linkages in acidic environments. The optimum pH of gamma - amylase is 3.0

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water

- Micropipet and other basic laboratory equipment.

- MEASURE Multi Calibrator and MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: α-glucosidase

Reagent R-1 is ready for use

- Reagent R-2: ethylidene-4-nitrophenol-G7

(maltoheptaose) ethylidene-G7 (maltoheptaose)-PNP}

Reagent R-2 is ready for use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrator MEASURE Multi Calibrator (separately sold): Put 5 mL of purified water to the vials of Calibrator (MEASURE Multi Calibrator), leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, Calibrator can be used without dilution.

- Controls MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2 (separately sold): Put 5 mL of purified water to the vials of controls (Lyo L-1 and Lyo L-2); leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, controls can be used without dilution.

5. SAMPLE PREPARATION & STORAGE

- Serum: Wait until the sample is completely coagulated. Take the supernatant to use as specimens.

- Plasma: Treat sample by anticoagulant (Li-heparin and K2-EDTA); leave sample to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take the plasma layer (supernatant) and use as specimens.

- Analyze sample soon after collection

- Stability in serum or plasma

  - 7 days at 15 - 25°C
  - 30 days at 2 - 8°C

- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

α-Amylase reacts with substrate ethylidene-4- nitrophenol-G7 (maltoheptaose) [ethylidene-G7 (maltoheptaose)-PNP] and isolates nitrophenol compounds.

When α-glucosidase coexists with this reaction system, p-nitrophenol (PNP) is generated by reaction. Activity of α-amylase can be obtained by measuring change of absorbance of PNP.

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[280s]{37^\circ C}$	Reagent 2
6µL		150µL		30µL
$\xrightarrow[125s]{37^\circ C}$		Meas. Abs I	$\xrightarrow[195s]{37^\circ C}$	Meas. Abs II
		(405/660nm)		(405/660nm)

⟶ ΔAbs at 405/660nm ⟶ Activity of AMY

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD.** for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank

- Plot a calibration curve AMY = f(ΔAbs)

- Calculate AMY in specimen using the curve

(doing same procedure for Controls)

Unit conversion

U/L x 0.0167 = µkat/L

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an AMY enzyme activity range of 2 - 2000 U/L.

- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Detection Limit

Limit of Blank (LoB)	=	0.5 U/L
Limit of Detection (LoD)	=	2.0 U/L
Limit of Quantitation (LoQ)	=	2.0 U/L

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: Change in absorbance when measuring purified water as sample ranges from 0.00 to 0.01. Change in absorbance is 0.035 - 0.100 Abs/min when measuring a standard solution of known amylase activity of 100 - 200 U/L as sample.

- Accuracy: When measuring a control sample, the result is within ±10% of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using controls followed the CLSI Approved Guidline EP5-A2 with repeatability, reproducibility and total precison (1 aliquot per run, 2 run per day, 20 days).The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 3% and Total Precision is less than 5%.

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
Control Lyo L-1	104.0	1.03	0.99
Control Lyo L-2	339.3	1.66	0.49

Reproducibility	*Mean U/L*	*SD U/L*	*CV %*
Control Lyo L-1	104.0	1.52	1.46
Control Lyo L-2	339.3	4.98	1.47

Total precision	*Mean U/L*	*SD U/L*	*CV %*
Control Lyo L-1	104.0	1.68	1.62
Control Lyo L-2	339.3	5.12	1.51

e. Corelation Test

Serum

Regression equation: y = 1.0067x - 1.5160 (n = 61)

Correlation coefficient r = 0.9995

Urine

Regression equation: y = 0.9996x – 2.5794

Correlation coefficient r = 0.9998

Reference material for calibration

ReCCS JCCLS CRM-001

10. EXPECTED VALUES

Serum/Plasma 44 - 132 U/L

Urine 50 - 500 U/L

Amylase/Creatinine Clearance Ratio (ACCR)

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$ACCR\ [\%] = \frac{Urine\ Amylase\ \left[\frac{U}{L}\right] x\ Serum\ Creatinine\ \left(\frac{mg}{L}\right)}{Serum\ Amylase\ \left(\frac{U}{L}\right) x\ Urine\ Creatinine\ \left(\frac{mg}{L}\right)} x\ 100$$

The ACCR is approximately equal to 2 - 5 %.

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated bilirubin concentration up to 40 mg/dL and free bilirubin concentration up to 40 mg/dL

- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL

- Lipemia (Intralipid): No significant interference triglycerides concentration up to 3000 FTU

- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

- Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

- Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		Rate
Temperature		37°C
Volume (µL)	Specimen	6
	R1	150
	R2	30
Wavelength (nm)	Main	405
	Sub	660
Measurement (cycle)	Point 1	10
	Point 2	24
	Point 3	34
Calibration type		Linear
Unit		U/L

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.

- Perform the QC procedure on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11A013A	1x60mL; 1x12mL	280	540
11A013A2	2x60mL; 2x12mL	560	1080
11A013A3	3x60mL; 3x12mL	840	1620
11A013A4	4x60mL; 4x12mL	1120	2160
11A003A	5x60mL; 5x12mL	1400	2700
11A013A6	6x60mL; 6x12mL	1680	3240
11A013	1x90mL; 1x18mL	420	810
11A013-2	2x90mL; 2x18mL	840	1620
11A003	3x90mL; 3x18mL	1260	2430
11A013-4	4x90mL; 4x18mL	1680	3240
11A013-5	5x90mL; 5x18mL	2100	4050

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series; Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;…

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. Kara Rogers, in The Editors of Encyclopaedia Britannica (Third Edition), 2020
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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