AMYLASE IFCC
Multi-Purpose (MPR)
Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL183A	5x100 ml	AMYLASE - 1	2-8°C
	1x100 ml	AMYLASE - 2	
GL193A	5 x 50 ml	AMYLASE - 1	
	1 x 50 ml	AMYLASE - 2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of amylase in serum, urine and plasma, based upon the IFCC recommendations, on automated and semi-automated analysers.

SUMMARY AND EXPLANATION: [1]

Two types of amylase are present in human serum, salivary (type S) and pancreatic (type P). While type P is attributed almost totally to the pancreas, type S is found in a number of other tissues. The measurement of amylase is most widely used in the diagnosis of acute pancreatitous, where levels can be 50 times the normal value. Increased levels are also found renal failure, pulmonary inflammation, disease of the salivary gland and macroamylasemia.

PRINCIPLE OF THE TEST: [1]

Amylase cleaves the substrate ethylidine (G_7) p-nitrophenyl 2-D maltoheptaside. The G_2 PNP, G_3 PNP and G_4 PNP are completely hydrolysed to p-nitrophenol and glucose by glucosidase.

$$5\ \text{ethylidene-G}_7\text{PNP} + 5\ H_2O \xrightarrow{\alpha-\text{amylase}} 2\ \text{ethylidene-G}_5 + 2\ G_2\text{PNP} + 2\ \text{ethylidene-G}_4 + 2\ G_3\text{PNP} + \text{ethylidene-G}_3 + G_4\text{PNP}$$

$$2\ G_2\text{PNP} + 2\ G_3\text{PNP} + G_4\text{PNP} + 14\ H_2O \xrightarrow{\alpha-\text{glucosidase}} 5\ \text{PNP} + 14\text{G}$$

PNP = p-nitrophenol. G = glucose

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Both reagents: Clear, colourless liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
This product is not hazardous under EU specifications. Contains Sodium Azide. Material Safety Data Sheet is available upon request.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:
Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	*Ingredients*	*Concentration in Tests*
Reagent 1	Hepes buffer pH 7.25	52 mmol/l
	Sodium Chloride	87 mmol/l
	Magnesium Chloride	12.6 mmol/l
	Calcium Chloride	0.075 mmol/l
	α-Glucosidase	4000 U/l
	PRESERVATIVES	---
Reagent 2	Hepes Buffer pH 7.15	52 mmol/l
	4,6-Ethylidene-G7-PNP	22 mmol/l
	PRESERVATIVES	---

REAGENT PREPARATION AND STABILITY:

Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label.

Monoreagent procedure: Add 1 volume of Reagent 2 to 5 Volumes of Reagent 1.
Working reagent is stable 10 days at 2-8°C.
Bireagent procedure: Liquid reagent 1 and 2 are ready for use.
Once open, components are stable until expiry date on label if store and handle properly.

TYPE OF SPECIMEN: [1]

Use serum or heparin/EDTA plasma or urine as specimen.

It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification.
- Serum/plasma should be separated from cells within 8 hours after collection.
  Stability[2]: up to 1 month at 2-8°C.
- Collect urine without additives. Dilute 1:3 with deionised water. Multiply by dilution factor to recover patient's results. *Stability*[3]: up to 10 days at 2-8°C.

TEST PROCEDURE:

Materials required but not supplied:

Description	*Catalog. No.*	*Description*	*Catalog. No.*
General Chemistry Calibrator	GL983	Photometer	N/A
General Chemistry Control Level 1	GL922	General Laboratory Equipment	N/A
General Chemistry Control Level 2	GL932		

Assay procedure:
Wavelength: λ: 405 nm
Temperature: 30°C or 37°C
Optical path: 1 cm light path.

MONOREAGENT PROCEDURE:	**Blank**	**Calibrator**	**Sample**
Working reagent	1000 µl	1000 µl	1000 µl
Sample	----	----	30 µl
Calibrator	----	30 µl	----
Gently mix and Incubate at 30°C or 37°C for 3 minute, then measure the change of Optical Density per minute (ΔOD/min) over the next 3 minutes.			

BIREAGENT PROCEDURE:	**Blank**	**Calibrator**	**Sample**
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	30 µl
Calibrator	----	30 µl	----
Gently mix and Incubate at 37°C for 5 minutes			
Reagent 2	200 µl	200 µl	200 µl
Gently mix and Incubate at 37°C for 3 minutes, then measure the change of Optical Density per minute (ΔOD/min) over the next 4 minutes			

Enzyme Calibration:
K-factor should be determined using fresh calibrator.
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory Q.C. Programme.

CALCULATION:

$$\text{Concentration} = \frac{\Delta Abs/\min_{Sample}}{\Delta Abs/\min_{Calibrator}} \times \text{Concentration of Calibrator}$$

(Conversion factor: Qty in µKat/l = Qty in U/l x 0.0167).

EXPECTED VALUES: [5]

	U/l	*µkat/l*
Serum/plasma*	28 to 100	0.47 – 1.67
Urine	460	7.67

*EDTA plasma values are approximately 8% lower than serum values
Each laboratory should establish its own reference range. Amylase results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 1988 U/l (33 µkat/l).
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Interfering substances:
Results of study are as follows:

Bilirubin (mixed isomers): Less than 10% interference up to 600 µmol/l Bilirubin
Haemolysis: Less than 10% interference up to 5 g/l Haemoglobin
Lipemia: Less than 10% interference up to 5 g/l Lipemia

Sensitivity:
The Lower Detectable Level was estimated at 1.0 U/l (0.016 µkat/l).

Precision:

Within Run N = 20	Mean *(U/l)*	*SD*	*% CV*	*Between Run N = 20*	Mean *(U/l)*	*SD*	*% CV*
Level 1	159.5	1.02	0.64	Level 1	160.2	0.96	0.60
Level 2	417.5	2.42	0.58	Level 2	416.4	3.84	0.92

Method Comparison:
Using 50 samples, a comparison, between this Amylase IFCC test (y) and another commercially available test (x), gave the following results:

y = 0.930x – 3.067	r = 1.000	Sample range: 30 to 2002 U/l

BIBLIOGRAPHY:

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5. Junge W, Waldenstrom J, Bouman A et al. Evaluation of the Assays for Total and Pancreatic α-Amylase based on 100% Cleavage of ET-G7-PNP at 6 European Clinical Centres (Poster Medlab 97). Basel, Switzerland; 12 th IFCC European Congress of Clinical Chemistry, August 17-22, 1997.

SYMBOLS:
The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard

CE Mark - Device comply with the Directives 98/79/EC

Storage temperature → Reconstitute with
Expiry Date (Last day of the month) — Manufactured By
Biological risk — Consult Instruction for Use

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