ALT IFCC Multi-Purpose (MPR) Liquid Reagent

KIT SPECIFICATIONS:

Cat. No.	*Quantity*	*Reagent*	*Storage*
GL732AL	10 x 15 ml 2 x 15 ml	ALT IFCC - 1 ALT IFCC - 2	2 - 8°C
GL742AL	5 x 50 ml 1 x 50 ml	ALT IFCC - 1 ALT IFCC - 2	2 - 8°C
GL752AL	5 x 100 ml 1 x 100 ml	ALT IFCC - 1 ALT IFCC - 2	2 - 8°C

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of Alanine Aminotransferase (ALT) in serum and plasma, based upon the IFCC recommendations, on automated and semi-automated analysers.

SUMMARY AND EXPLANATION:

Alinine amino transferase belongs to the transaminases that catalyse the inter-conversion of amino acids and keto acids by transfer of amino groups. Most of the enzyme activity is found in the liver yet significant concentrations are also found in kidney, heart, skeletal muscle and spleen. ALT measurements are almost exclusively used for the diagnosis of parenchymal liver disease. Elevated levels of this enzyme are often apparent prior to any clinical abnormality and levels of 100 times the upper limit of normal can be encountered in severe hepatic necrosis.

Pyridoxyl-5-Phosphate functions as a co-enzyme in the aminotransferase reaction. While most patient samples contain endogenous pyridoxyl phosphate the IFCC recommendation specify the addition of the co-enzyme to supplement pyridoxyl-5-phosphate deficient samples. The pyridoxyl-5-phosphate is available as a powder vial from Glenbio Ltd. Cat. No. GL806PP (12 x 50 ml) and GL816PP (6 x 66 ml).

PRINCIPLE OF THE TEST: [1,2]

This method is formulated in accordance with the kinetic determination of the alanine aminotransferase.

L-Alanine + α-Ketoglutarate $\xrightarrow{ALT}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H$^+$ $\xrightarrow{Lactate\ Dehydrogenase}$ L-Lactate + NAD+

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:
Reagent 1: Colourless liquid.
Reagent 2: Pale beige liquid.
Any significant changes from the above 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:
CAUTION: Take all necessary precautions required when handling laboratory reagents. Contains Sodium Azide. Material Safety Data Sheet is available upon request.

Label Elements:

WARNING

H315 Causes skin irritation.
H319 Causes serious eye irritation.

Precautionary Statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313 If skin irritation occurs: Get medical advice/attention.

Handling precautions:
- 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	TRIS Buffer pH 7.3	125.0 mmol/l
	L-Alanine	625.0 mmol/l
	LDH	1500 U/l
	STABILISERS & PRESERVATIVES	---
Reagent 2	α-Ketoglutarate	94 mmol/l
	NADH	0.23 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 5 weeks 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 handled properly.

TYPE OF SPECIMEN: [1,2]

Use serum or heparin/EDTA plasma, free of haemolysis, 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: up to 24 hours at 2-8°C$_3$.

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: 340 nm (Hg 334-Hg 365)
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	----	----	100 µl
Calibrator	----	100 µl	----
Gently mix and Incubate at 30°C / 37°C for 1 minute, then measure the change of Optical Density per minute (ΔOD/min) over a further 3 minutes.			

Factor Calculation:
340 nm: U/l = ΔOD/min x 1746 334 nm: U/l = ΔOD/min x 1780 365 nm: U/l = ΔOD/min x 3235

BIREAGENT PROCEDURE:	**Blank**	**Calibrator**	**Sample**
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	100 µl
Calibrator	----	100 µl	----
Gently mix and Incubate at 37°C for 2 minutes			
Reagent 2	200 µl	200 µl	200 µl
Gently mix and Incubate at 37°C for 1 minutes, then measure the change of Optical Density per minute (ΔOD/min) over a further 4 minutes.			

Factor Calculation:
340 nm: U/l = ΔOD/min x 2063 334 nm: U/l = ΔOD/min x 2103 365 nm: U/l = ΔOD/min x 3823
* The above factors should be validated using General Chemistry Calibrator (GL973).

Enzyme Calibration:
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 programme.

Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the Laboratory Q.C. Programme.

CALCULATION:

340 nm: U/l = ΔOD/min x 1746 334 nm: U/l = ΔOD/min x 1780 365 nm: U/l = ΔOD/min x 3235
(Conversion factor: Qty in µKat/l = Qty in U/l x 0.0167).

EXPECTED VALUES[2]:

In Serum, with P-5'-P at 37°C	*Values U/l*	*Values µkat/l*
Adult man	13 to 40	0.22 – 0.68
Adult woman	10 to 28	0.17 – 0.48
Infant	13 to 45	0.22 – 0.77

Each laboratory should establish its own reference range. ALT results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

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

Linearity:
Linear up to 418 U/l (7 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:
Bilirubin (mixed isomers): Less than 10% interference up to 600 µmol/l Bilirubin.
Haemolysis: Less than 10% interference up to 5g/l Haemoglobin.
Lipemia: Less than 10% interference up to 5g/l Intralipid.

Sensitivity:
The Lowest Detectable Level was estimated at 3 U/l (0.05 µkat/l).

Precision:

Within Run N = 20	Mean *(U/l)*	*SD*	*% CV*	*Between Run N = 20*	Mean *(U/l)*	*SD*	*% CV*
Level 1	35	1.40	4.02	Level 1	36	1.48	4.12
Level 2	141	2.15	1.52	Level 2	138	2.69	1.96

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

y = 0.999x – 1.370	r = 0.999	Sample range: 6 to 280 U/l

BIBLIOGRAPHY:

1. Z. Klin. Biochem. 1970; 8:658
2. Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54 and 352-390 and 962.
3. Fischbach F, Zawta B. Age-dependent Ref Limits of Several Enz in Plasma at Dif Measuring Temp. Klin Lab 1992; 38:555-561.
4. Zawta B, Klein G, Bablok W. Temperature Conversion in Clinical Enzymology, Klin Lab 1994; 40:33-42.
5. IFCC Scientific Committee Clin. Chem. Biochem. 1980; 18:521-534.

SYMBOLS:

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

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	CAL	Calibrator
CE	CE Mark - Device comply with the Directives 98/79/EC		
	Storage temperature		Consult Instruction for Use
	Expiry Date (Last day of the month)		Manufactured By

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