ALT/GPT

Cat. No.	Pack Name	Packaging (Content)
BLT00052	ALT/GPT 250	R1: 4 x 50 ml, R2: 1 x 50 ml
BLT00053	ALT/GPT 500	R1: 4 x 100 ml, R2: 1 x 100 ml

EN

CE IVD

INTENDED USE
Diagnostic reagent for quantitative *in vitro* determination of ALT/GPT (Alanine Aminotransferase) in human serum and plasma.

CLINICAL SIGNIFICANCE
ALT/GPT is present in high concentration in liver and to a lesser extent in kidney, heart, skeletal muscle, pancreas, spleen and lung. Increased levels of ALT/GPT however is generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, viral or toxic hepatitis and obstructive jaundice. Characteristically ALT/GPT is generally higher than AST/GPT in acute viral or toxic hepatitis, whereas for most patients with chronic hepatic disease, ALT/GPT levels are generally lower than AST/GPT levels. Elevated ALT/GPT levels have also been found in extensive trauma and muscle disease, circulatory failure with shock, hypoxia, myocardial infarction and haemolytic disease.

PRINCIPLE
This ALT/GPT reagent is based on the recommendations of the IFCC without pyridoxal phosphate. The series of reactions involved in the assay system is as follows:

L-Alanine + 2-oxoglutarate $\xrightarrow{\text{ALT/GPT}}$ Pyruvate + L-Glutamate

Pyruvate + NADH $\xrightarrow{\text{LDH}}$ L-Lactate + NAD^+

Sample pyruvate + NADH $\xrightarrow{\text{LDH}}$ L-Lactate + NAD

1. The amino group is enzymatically transfered by SGPT / ALAT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.

2. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due the oxidation of NADH.

3. Endogenous sample pyruvate is rapidly and completely reduced by LDH during initial incubation period to avoid interference during the assay.

REAGENT COMPOSITION
R1

Tris Buffer (pH 7.5)	137.5 mmol/l
L-Alanine	709 mmol/l
LDH (microbial)	≥ 2000 U/l

R2

CAPSO	20 mmol/l
2-oxoglutarate	85 mmol/l
NADH	1.05 mmol/l

REAGENT PREPARATION
Reagents are liquid, ready to use.

STABILITY AND STORAGE
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Two reagents method – substrate start
Reagents are ready to use. After the first opening the vials, reagents are stable for 30 days at 2–8°C in the dark.
Monoreagent method – sample start
Mix 4 portion of reagent R1 with 1 portion of reagent R2.
Stability: 5 days at 20–25 °C in the dark
4 weeks at 2–8 °C in the dark

SPECIMEN COLLECTION AND HANDLING
Use unheamolytic serum or plasma (EDTA, heparin).
It is recommended to follow NCCLS procedures (or similar standardized conditions).
Loss of activity: within 3 days at 2–8 °C < 10 %
within 3 days at 15–25 °C < 17 %
Stability at least 3 months at -20 °C.
Discard contaminated specimens.

CALIBRATION
Calibration with the calibrator XL MULTICAL, Cat. No. XSYS0034 is recommended.

QUALITY CONTROL
For quality control ERBA NORM, Cat. No. BLT00080 and ERBA PATH, Cat. No. BLT00081 are recommended.

UNIT CONVERSION
U/l x 0.017 = µkat/l

EXPECTED VALUES [4]
At 37°C Men up to 45 U/l
Women up to 34 U/l
It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

PERFORMANCE DATA
Data contained within this section is representative of performance on ERBA XL systems. Data obtained in your laboratory may differ from these values.
Limit of quantification: 4.4 U/l
Linearity: 360 U/l
Measuring range: 4.4 – 360 U/l

PRECISION

Intra-assay precision Within run (n=20)	Mean (U/l)	SD (U/l)	CV (%)
Sample 1	58.32	2.52	4.31
Sample 2	114.72	1.38	1.20

Inter-assay precision Run to run (n=20)	Mean (U/l)	SD (U/l)	CV (%)
Sample 1	40.20	1.20	3.12
Sample 2	112.8	2.40	1.95

COMPARISON
A comparison between XL-Systems ALT/GPT (y) and a commercially available test (x) using 40 samples gave following results:
y = 0.979 x - 1.8 U/l
r = 0.996

INTERFERENCES
Following substances do not interfere:
haemoglobin up to 2.5 g/l, bilirubin up to 30 mg/dl, triglycerides up to 2000 mg/dl.

WARNING AND PRECAUTIONS
For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.
Reagents of the kit are not classified like dangerous but contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

WASTE MANAGEMENT
Please refer to local legal requirements.

ASSAY PROCEDURE
Wavelength 340 nm, Hg 334 nm, Hg 365 nm
Cuvette 1 cm
Two reagents method – substrate start

Reagent 1 (buffer)	1.000 ml
Sample	0.100 ml

Mix and incubate for 5 min. at 37°C. Then add:

Reagent 2 (substrate)	0.250 ml

Mix, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

Monoreagent method – sample start

Working solution	1.000 ml
Sample	0.100 ml

Mix, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

CALCULATION
1. $\text{ALT/GPT (U/l)} = \frac{\Delta A_{sam}/\text{min}}{\Delta A_{cal}/\text{min}} \times C_{cal}$ C_{cal} = calibrator concentration

2. Using factor:
ALT/GPT (U/l) = ΔA/min x f f = factor

Factors:	Substrate Start:	25° or 30°C	37°C
	Factor at 340 nm	1151	2143
	Factor at 334 nm	1173	2184
	Factor at 365 nm	2132	3971
	Sample Start:	**25° or 30°C**	**37°C**
	Factor at 340 nm	952	1745
	Factor at 334 nm	971	1780
	Factor at 365 nm	1765	3235

Applications for automatic analysers are available on request.

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N/40/13/A/INT Date of revision: 27. 11. 2013

ASSAY PARAMETERS FOR PHOTOMETERS

Mode	Kinetic
Wavelength 1 (nm)	340
Sample Volume (µl)	50/100
Working Reagent Volume (µl)	500/1000
Lag time (sec.)	60
Kinetic interval (sec.)	60
No. of readings	3
Kinetic factor	1745
Reaction temperature (°C)	37
Reaction direction	Decreasing
Normal Low (U/l)	0
Normal High (U/l)	34
Linearity Low (U/l)	4.4
Linearity High (U/l)	360
Blank with	Water
Absorbance limit (max.)	1.1
Units	U/l

REFERENCES

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55-65.

2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.

3. Schumann G, Bonora R, Ceriotti F, Férard G et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 5: Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med 2002;40:725-33.

4. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER, Fifth Edition, 2012.

SYMBOLS USED ON LABELS

REF Catalogue Number

Manufacturer

See Instruction for Use

LOT Lot Number

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

Storage Temperature

Expiry Date

IVD In Vitro Diagnostics

CONT Content

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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