BIOLABO
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MANUFACTURER:
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ALT GPT (IFCC) Single vial

Reagent for quantitative determination of Alanine amino transferase activity (ALT) [EC 2.6.1.2] in human serum or plasma.

REF 80027	**R1**: 20 X 10 mL	REF 80127	**R1**: 8 x 30 mL
REF 80227	**R1**: 10 x 125 mL	REF 80327	**R1**: 6 x 200 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

ALT is present in very high amounts in liver and kidney, and in smaller amounts in skeletal muscle and heart. Although serum levels of both AST and ALT become elevated whenever diseases process affecting liver cells integrity, ALT is the more liver-specific enzyme. Serum elevations of
ALT activity is rarely observed in conditions other than parenchymal liver disease (cirrhosis, carcinoma, hepatitis, obstructive jaundice or liver stroke). Moreover its elevation persists longer than do those of AST activity. Measurement of both AST and ALT has some value in distinguishing hepatitis from other parenchymal lesions.

PRINCIPLE (4) (5) (6)

Method developed by Wrobleski and LaDue, optimised by Henry and Bergmeyer (following modified IFCC recommendations). Reaction scheme is as follows:

L- Alanine + 2-Oxoglutarate $\xrightleftharpoons{ALT}$ Pyruvate + L-Glutamate

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

The decrease in absorbance due to the conversion of NADH into NAD^+, and proportional to ALT activity in the specimen, is measured at 340 nm.

Absence of P_5P allows a better stability of working reagent.

REAGENTS COMPOSITION

Vial R1 **WORKING REAGENT**

2-Oxoglutarate	15	mmol/L
L-Alanine	500	mmol/L
LDH	≥ 1600	UI/L
NADH	≤ 0.18	mmol/L
Tris Buffer	100	mmol/L
pH at 30°C	7.50 ± 0.1	
Preservative		

Before reconstitution: Xn, Harmful
R22-32: Harmful if swallowed. Contact with acid libaerates very toxic gas.
S22-S28$_1$: Do not breath dust. After contact with skin, rinse immediately with plenty of water

Once reconstituted: None

REAGENTS PREPARATION

REF 80027: Use a non-sharp instrument to remove aluminium cap. Add promptly to the contents of the vial the amount of demineralised water stated on the label.

Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8°C

- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 60 days when free from contamination.
- Discard any reagent if cloudy or if absorbance measured at 340 nm is < 1.000.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2) (7)

Unhemolysed serum. Do not use heparinised plasma.

ALT is stable in serum or plasma for:

- 24 hours at room temperature.
- 7 days at 2-8°C.

INTERFERENCES (3) (6)

Hemoglobin: No interference up to 300 µmol/L Hb.
Hemolysis: Positive interference due to ALT released from erythrocytes.
Bilirubin: No interference up to 20 mg/dl (342 µmol/L).
Turbidity: No interference up to 7.00 mmol/L triglycerides.

LDH contained in reagent allows, during pre-incubation step, the reduction of endogenous pyruvate which would positively interfere.

Elevated ALT level may involve NADH depletion during pre-incubation stage, which may lead to under-estimated results. In case of lipemic or icteric specimens, increased absorbance may mask this phenomenon.

It's recommended to check these specimens diluted (1 + 4) in saline solution.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment.
2. Normal and pathological control sera.
3. Demineralised water for reagent preparation.

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

- Use the theoretical calibration factor (§ **CALCULATION**)
- Or REF 95015 BIOLABO Multicalibrator (calibration value determined with validated statistical technics and metrologically controlled instrument)
- or a multiparametric calibrator traceable to a reference method or material

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010
  BIOLABO EXATROL-P Level II REF 95011
- Other assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

UI/L	at 30°C	at 37°C
Newborns, Infants	9-32	13-45
Men	7-28	10-40
Women	5-25	7-35

Each laboratory should establish its own normal ranges for the population it serves.

PERFORMANCE CHARACTERISTICS

Within-run N = 30	*Normal level*	*High level*
Mean IU/L	32	141
S.D. IU/L	1.06	1.94
C.V. %	3.3	1.4

Beetwen run N = 33	*Normal level*	*High level*
Mean IU/L	39	98
S.D.IU/L	1.15	1.45
C.V. %	2.9	1.5

Detection limit: approximately 7 IU/L

Sensitivity for 17 IU/L: approximately 0. 010 ΔAbs/min at 340 nm.

Comparison studies with commercially available reagent:

y = 0.9813 x – 0.6606 r = 0.9983

LINEARITY

The assay is linear up to 350 IU/L.

If ΔAbs./min > 0.200, reduce specimen volume or dilute specimen with saline solution and reassay taking into account the dilution factor to calculate the result. Linearity will depend on the specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into 1 cm path length thermostated cuvette:	
Reagent	1 mL
Bring to 37°C (30°C) then add:	
Specimen	100 µL

Mix. Start a timer. Record initial absorbance after 1 minute at 340 nm.
Record the absorbance again every minutes during 3 minutes.

Calculate absorbance change per minute (ΔAbs/min).

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

With theoretical factor:

IU/L = (ΔAbs/min) x 1746

$$\mu Kat/L = \frac{IU/L}{60}$$

With seric multicalibrator:

$$ALT\ Activity = \frac{(\Delta Abs/min)\ Assay}{(\Delta Abs/min)\ Calibrator} \times Calibrator\ Concentration$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 652-657*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 64-67*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-6 to 3-16.*
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(5) *Bergmeyer HU.,and al. Clin. Chem. (1978), 24, p.58-73*
(6) *IFCC Method for L-Alanine aminotransferase. J Clin. Chem., Clin. Biochem.(1986), 24, p.481-495).*
(7) *MURRAY RL., « Alanine aminotransironase » in clinical chemistry. Theory, analysis, and correlation.Kapan LA, Pesce AJ, (Eds), CV Mosby St Louis (1984): 1090*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with