BIOLABO
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AST TGO-ALT TGP

Colorimetric Method

Reagent for quantitative determination of Alanine amino transferase (ALT) [EC 2.6.1.2] and Aspartate amino transferase (AST) [EC 2.6.1.1] activities in human serum and plasma

TGO	REF 92025	**R1** 1 x 100 mL	**R3** 1 x 100 mL	**R4** 1 x 10 mL
TGP	REF 92027	**R2** 1 x 100 mL	**R3** 1 x 100 mL	**R4** 1 x 10 mL
	REF 92026	NaOH Solution 0,4 N		**R1** 1 x 500 mL (ordered separetely)

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 processes affecting liver cells integrity, ALT is the more liver-specific enzyme. Serum elevations of ALT activity are 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.

AST is distributed in all body tissues, but greatest activity occurs in liver, heart, skeletal muscle and in erythrocytes. Minimal activity occurs in skin, kidney and pancreas. Although serum levels of both AST and ALT become elevated whenever diseases processes affecting liver cells integrity (viral hepatitis, liver necrosis, cirrhosis), an increased AST activity in serum or plasma appears in more than 97% of cases of myocardial infarction. AST levels (and occasionnaly ALT) are also elevated in progressive muscular dystrophy, pulmonary emboli, acute pancreatitis…

PRINCIPLE (4)

Colorimetric method developed by Tonhazy, White, and Umbreit and adapted for the determination of the activity in serum by Reitman and Frankel. Reaction scheme is as follows :

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

L-Aspartate + 2-Oxoglutarate $\xrightleftharpoons{AST}$ Oxaloacetate + L-Glutamate

Then, Pyruvate or Oxalate reacts with 2, 4 DNPH to form 2, 4 Dinitrophenylhydrazones, which absorbance at 505 nm in alcaline solution is proportional to AST or ALT activity in the reactional mixture.

REAGENTS

Vial R1 — TGO SUBSTRATE

Phosphate Buffer pH 7.5	85 mmol/L	2-oxoglutarate	2 mmol/L
L-aspartate	200 mmol/L	Preservative	

Vial R2 — TGP SUBSTRATE

Phosphate Buffer pH 7.5	100 mmol/L	2-oxoglutarate	2 mmol/L
L-alanine	200 mmol/L	Preservative	

Vial R3 — COLORATION REAGENT

2,4-dinitrophenyl-hydrazine (DNPH)	1	mmol/L
HCl	1	mol/L

Xi, R36/38 : Irritating to eyes and skin
S37/39 : Wear suitable gloves and eyes/face protection

Vial R4 — STANDARD SOLUTION

Sodium Pyruvate	2	mmol/L
Sodium Mercurothiolate	0.1	%
Phosphate Buffer pH 7.5	100	mmol/L
Preservative		

Xn, R20/21/22 : Harmful by inhalation, in contact with skin and if swallowed
S37-S38 : Wear suitable gloves. In case of insufficient ventilation wear suitable respiratory equipment

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 or 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.

REAGENTS PREPARATION

Reagents are ready for use.

Prepare NaOH solution 0.4 N diluting 16 g NaOH into 1 liter of demineralised water. This solution is irritating (see § **SAFETY CAUTIONS**).

STABILITY AND STORAGE

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

- Without contamination, when stored and used as described in the insert, reagents are stable until expiry date stated on the label.
- Standard Solution (vial R4) : transfer requested quantity, recap and store at 2-8°C.

Discard reagents if cloudy or if reagent blank at 505 nm is > 0.400.

Don't use reagents after expiry date stated on the label of the vial.

SPECIMEN COLLECTION AND HANDLING (2)

Serum or heparinized plasma, unhemolized.

ALT activity is stable in the specimen for :

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

AST activity is stable in the specimen for :

- 24 hours at room temperature.
- 28 days at 2-8°C.
- at least 1 year at –20°C.

The addition of Phosphate pyridoxal (0.1 mM) preserves AST activity in the specimen for 7 days at room temperature.

INTERFERENCES (3)

Hemolysis : Positive interference because of ALT contained in erythrocytes.

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

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.
3. REF 92026 : NaOH 0.4 N Solution (§ **REAGENTS**)

CALIBRATION

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

It is recommended to establish a new Standard Curve when using a new batch of reagent (§ **CALCULATION**) or to refer to the **enclosed Standard Curve (batch specific)**.The value of the standard has been determined under metrological control, by weighing on analytical balance.

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 (1) (2)

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

AST (IU/L)	at 30°C	at 37°C
Newborn	25-75	39-117
Infant	15-60	23-94
Adult	8-20	13-31

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

PERFORMANCE CHARACTERISTICS

	Within-run N = 20		*Beetwen run N = 20*	
TGO Color.	***Normal level***	***High level***	***Normal level***	***High level***
Mean IU/L	37.7	167	38	144
S.D. IU/L	1.1	9.4	3.75	13.5
C.V. %	2.9	5.6	9.9	9.3
TGP Color.	***Normal level***	***High level***	***Normal level***	***High level***
Mean IU/L	51.6	90.6	29.7	92
S.D. IU/L	2.2	2.5	1.71	8.2
C.V. %	4.2	2.8	5.8	8.9

Detection limit : approximately 7.2 IU/L

Sensitiviy for 100 IU/L : approximately 0.200 Abs at 505nm.

Comparison study with commercially available reagent :

92027 : y = 1,0477 x - 2,3 r = 0,9737

92025 : y = 0,8984 x + 3,6 r = 0,9729

LINEARITY

Above 225 IU/L, dilute specimen (1 + 9) with saline solution and re-assay multiplying the result obtained on the Standard Curve by 10 (dilution factor). Linearity limit depend on the specimen/reagent ratio.

MANUAL PROCEDURE

Board 1 : Standard Curves establishment.

Let stand reagents and specimens at room temperature.

Pipette into test tubes (**mL**) :

Tube number	**1**	**2**	**3**	**4**	**5**	**6**
Demineralised water	0.200	0.200	0.200	0.200	0.200	0.200
R1 or R2	1	0.9	0.8	0.7	0.6	0.5
R4 (Standard)	--	0.1	0.2	0.3	0.4	0.5
R3 (Colorant)	1	1	1	1	1	1
Mix. Let stand for 20 minutes at room temperature. Add:						
NAOH 0.4 N	10	10	10	10	10	10
Mix. Let stand 5 minutes and read absorbances at 505 nm against water.						
TGO Units	0	30	70	135	225	350
TGP Units	0	40	80	140	225	325
There's no need to plot a new curve at each determination.						

Board 2 : Assays.

Let stand reagents and specimens at room temperature.

Pipette into test tubes	**TGO**	**TGP**
Reagent R1	1 mL	
Reagent R2		1 mL
Incubate for 5 minutes at 37°C. Add :		
Serum	200 µL	200 µL
Mix and incubate at 37°C during :	**Exactly 1 hour**	**Exactly 30 minutes**
Reagent R3	1 mL	1 mL
Mix and let stand 20 minutes at room temperature. Add:		
NaOH 0.4 N	10 mL	10 mL
Mix. Let stand 5 minutes and read absorbances at 505 nm against water.		

Notes :

1- Volumes may be reduced proportionaly without modifying results.

2- Blank Reagent : Replace serum by demineralised water in board

CALCULATION

Calculate the result as follows :

✓ **Refer to enclosed Standard Curves (batch specific)**

or

✓ Plot Standard Curves on millimeter paper (Absorbances) or semi-log (% of transmission) handling as indicated in board 1.

Abscissa : number of units (IU/L)

Ordinate : Absorbances (or % of transmission)

Transfer "Assay" absorbances or % of transmission on Standard Curve and calculate TGO or TGP activity in IU/L.

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 et p.76-77.*

(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-6 to 3-17 and p.3-68 to 3-79.*

(4) *A colorimetric method for the determination of serum GOT and GPT, REITMAN S. and FRANKEL S., Amer. J. Clin. Path., 1957; 28.p.56-63*

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