BIOLABO
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MANUFACTURER:
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AST GOT (IFCC) Single vial

Reagent for quantitative determination of Aspartate amino transferase activity [EC 2.6.1.1] in human serum and plasma

REF 80025	**R1** 20 X 10 mL	REF 80125	**R1** 8 x 30 mL
REF 80225	**R1** 10 x 125 mL	REF 80325	**R1** 6 x 200 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

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 and cirrhosis), an increased AST activity in serum or plasma appears in more than 97% of cases of myocardial infarction. AST levels (and occasionally ALT) are also elevated in progressive muscular dystrophy, pulmonary emboli, acute pancreatitis…

PRINCIPLE (4) (5)

Method developed by Karmen and Al, and optimised by Henry and al. (according to modified IFCC recommendations).

Reaction scheme is as follows:

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

Oxaloacetate + NADH + H^+ $\xrightleftharpoons{\text{MDH}}$ L-Malate + NAD^+

The decrease in absorbance proportional to AST activity in the specimen, is measured at 340 nm.
Absence of P_5P allows a better stability of working reagent.

REAGENTS COMPOSITION

R1	**AST (GOT) IFCC**	**Danger**
EDTA	5	mmol/L
2-Oxoglutarate	12	mmol/L
L-Aspartate	200	mmol/L
MDH	495	UI/L
LDH	820	UI/L
NADH	≤ 0.18	mmol/L
Tris Buffer	80	mmol/L
pH at 30°C	7.80 ± 0.1	
Preservative		

Before reconstitution:

Acute Tox. 2: H300 - Fatal if swallowed,
Aquatic Chronic 3: H412 - Harmful to aquatic life with long lasting effects

P264: Wash hands thoroughly after handling, P270: Do not eat, drink or smoke when using this product, P301+310: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician, P330: Rinse mouth, P501: Dispose of contents/container in accordance with dangerous waste disposal regulations. Classification due to Sodium Azide < 1 %. For more details, refer to Safety Data Sheet (SDS)

Once reconstituted, working reagent is not classified as dangerous

REAGENTS PREPARATION

- REF **80025** (Vial R1): Use a non-sharp instrument to remove aluminium cap.
- Other REF: Once opened, add promptly to the contents the amount of demineralised water indicated on the label.

  Mix gently until complete dissolution.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.

All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, stored and used as described, reagents are stable:

Unopened:

- Until expiry date stated on the label of the kit.

Once reconstituted:

- Working reagent (vial R1) is stable for 60 days when free from contamination.
- Discard reagent if cloudy or if absorbance at 340 nm is < 1.000.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum. Do not use heparinised plasma

AST is stable in serum or plasma for:

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

Adding pyridoxal phosphate (0.1 mM) improves stability at room temperature to 7 days.

LIMITS (3) (6)

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

Likewise oxaloacetate, product of the reaction, is carboxilated into pyruvate. This one will also be consumed by LDH contained in reagent and will not interfere with AST determination.

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. Demineralised water for reagent preparation
3. Thermostated Spectrophotometer or Biochemistry Analyzer

CALIBRATION

- REF 95015 BIOLABO Multicalibrator traceable to *ERM-AD457/IFCC*

The calibration frequency depends on proper instrument functions and on the preservation of reagent

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO EXATROL-P Level II
- 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)

UI/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 its own normal ranges for the population it serves.

PERFORMANCE at 37°C on KENZA 240TX

Linearity Range: between 5 and 310 IU/L

Detection limit: approx. 1.3 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	21.8	44.2	171.9
S.D. (IU/L)	0.6	0.7	2.7
C.V. %	2.5	1.6	1.6

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	22.5	45.3	176.9
S.D. IU/L	0.7	1.1	4.0
C.V. %	3.1	2.5	2.3

Comparison studies with commercially available reagent:

Realised on serum specimens (n=100) between 9 and 313 IU/L

y = 1.0265 x +0.9906 r = 0.9982

Analytical Sensitivity: approx. 0.0063 abs/min for 10 IU/L

Interferences:

Turbidity	No interference up to 0.133 abs
Total bilirubin	Negative interference from 399 µmol/L
Direct bilirubin	No interference up to 328 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1104 mg/dL
Haemoglobin	Positive interference from 109 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 1 month

Calibration Stability: 8 days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

PROCEDURE

Detailed Kenza 240TX procedure is available on request

Wavelength: 340 nm
Temperature: 37°C
Let stand reagents and specimens at room temperature.

	Automated analyzer	Manual procedure
Reagent	200 µL	1000 µL
Standard / Control or Specimen	20 µL	100 µL
Mix. Record initial absorbance after 1 minute at 340 nm. Record the absorbance again every minute during 3 minutes.		
Calculate absorbance change per minute (ΔAbs/min).		

Notes:

1- Performances and stability data's have been validated on KENZA 240TX and KENZA 450TX.

2-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user.

3- Applications proposal are available on request

CALCULATION

Calculate the result as follows:

With serum multicalibrator:

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

With theoretical factor:

Activity (IU/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{VR} \times 1000}{6.3 \times \text{VE} \times \text{P}}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
6.3 = Molar extinction coefficient for NADH at 340 nm
P = Pathlength (cm).

Example, with manual Procedure,
(Path length 1 cm, 37°C, 340 nm):

IU/L = (ΔAbs/min) x 1746

$$\text{µKat/L} = \frac{\text{IU/L}}{60}$$

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. 154-159*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-68 to 3-79*
(4) *HENRY R. J. and al., Am J clin Path (1960), 34, 381-398*
(5) *IFCC Method for L-Aspartate aminotransferase. J Clin. Chem. Clin. Biochem.(1986), 24, p.497-510.*
(6) *M. MATHIEU and col. SFBC. Comité de Standardisation. Recommandations pour la mesure de l'activité catalytique de l'Aspartate aminotransférase dans le sérum à 30°C. Ann. Biol. Clin. 1976. 34. 291-297*

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 | H2O Demineralized water | Biological hazard