BIOLABO
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MANUFACTURER:
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AST GOT (IFCC)Single vial

Reagent for quantitative determination of aspartate amino transferase activity (AST) [EC 2.6.1.1] in human serum or 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

IVD 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 due to the conversion of NADH into NAD^+, and 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

Vial R1 **WORKING REAGENT**

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: Xn, Harmful
R22-32: Harmful if swallowed. Contact with acid libaerates very toxic gas.
Once reconstituted: None
S22-S28$_1$: Do not breath dust. After contact with skin, rinse immediately with plenty of water.

REAGENTS PREPARATION

REF 80025: Use a non-sharp instrument to remove aluminium cap. Add promptly to the contents of the vial the amount of demineralised water indicated on the label.

Mix gently and wait for complete dissolution before using reagents (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 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.

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 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)

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.

INTERFERENCES (3) (6)

Haemoglobin: Positive interference above 150 µmol/L.
Haemolysis: Positive interference due to AST released from erythrocytes.
Turbidity: No interference.
Total bilirubin: Negative interference above 20 mg/dl.

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. 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 (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 CHARACTERISTICS

Within-run N = 30	*Normal level*	*High level*
Mean IU/L	31.6	161.1
S.D. IU/L	1.22	3.06
C.V. %	3.85	1.90

Beetwen run N = 33	*Normal level*	*High level*
Mean IU/L	34.4	179.3
S.D.IU/L	0.58	2.62
C.V. %	1.69	1.46

Detection limit: approximately 3 IU/L

Sensitivity for 17 IU/L: approximately 0.010 Abs/min at 340 nm.

Comparison studies with commercially available reagent:

y = 1.0265 x + 0.9906 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 serum multicalibrator:

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

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. 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