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# AST GOT Colorimetric Method

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

REF 92025 R1 1 x 100 mL R3 1 x 100 mL R4 1 x 10 mL

## TECHNICAL SUPPORT AND ORDERS

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Latest revision: www.biolabo.fr



Made In France

I: corresponds to significant modifications

## I INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).

It allows the quantification of global activity of the aspartate amino transferase (AST) enzyme in human serum and plasma to screen its level.

## GENERALITIES (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)

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:



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

## I REAGENTS

R1	GOT / AST	Substrate
		Phosphate Buffer pH 7.5 85 mmol/L
		L-Aspartate 200 mmol/L
		2-oxoglutarate 2 mmol/L
		Preservative

R3	GOT / AST	Dye
		2,4-dinitrophenyl-hydrazine (DNPH) 1,7 mmol/L
		HCl 1 mol/L
EUH210: Safety datasheet on request (HCL 2.5 - < 10%)		

R4	GOT / AST	Standard
		Sodium Pyruvate 2 mmol/L
		Sodium Mercurothiolate 0.1 %
		Phosphate Buffer pH 7.5 100 mmol/L
		Preservative

According to 1272/2008 regulation, these reagents are not classified as dangerous

## SAFETY CAUTIONS

- 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.
- Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

## REAGENTS PREPARATION

Ready for use.

## STABILITY AND STORAGE

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

- Unopened,
    - Until the expiry date stated on the label of the Kit.
  - Once opened:
    - Transfer requested quantity, well recap vials and store at 2-8°C,
    - Separated reagents are stable at least 6 months without contamination
- Discard reagents if cloudy or if reagent blank at 505 nm is > 0.400.

## 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)

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. REF 92026: NaOH 0.4 N
3. Spectrophotometer

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

## CALIBRATION

- **REF** 92025 (vial R4)  
or 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

- **REF** 95010 EXATROL-N Level I
- **REF** 95011 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. Prepare a fresh control serum and repeat the test
  2. If control is still out of range, use a new vial of fresh calibrator
  3. If control is still out of range, use a new vial of reagent and reassay
- If control is still out of range, please contact BIOLABO technical support or your local Agent.

## REFERENCE INTERVAL (2)

AST (IU/L)	at 37°C
New-born, Infants	39-117
Men	23-94
Women	13-31

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

## PERFORMANCE

On Spectrophotometer 37°C, 505 nm

Measuring Range: within Standard Curve limits

Detection limit: approximately 7.2 IU/L

Precision

	Intra-série N = 20		Inter-série N = 20	
	Taux normal	Taux élevé	Taux normal	Taux élevé
Moyenne UI/L	37,7	167	38	144,5
S.D. UI/L	1,1	9,4	3,75	13,5
C.V. %	2,9	5,6	9,9	9,3

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

Comparison study with commercially available reagent:

$$y = 0,8984 x + 3,6$$

$$r = 0,9729$$

Interferences:

Ascorbic acid	No interference up to 2500 mg/dL
Total bilirubin	Negative interference from 100 µmol/L
Haemoglobin	Positive interference from 90 µmol/L
Turbidity	Positive interference from 0.075 OD

Other substances may interfere (see § Limits)

Calibration frequency:

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).

## MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

### 1- STANDARD CURVE ESTABLISHMENT:

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
R2 (Substrate)	1	0.900	0.800	0.700	0.600	0.500
R4 (Standard)	--	0.100	0.200	0.300	0.400	0.500
R3 (Dye)	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.						
AST (IU/L)	0	30	70	135	225	350
There's no need to plot a new curve at each determination.						
See § Calibration and Quality Control						

### 1- ASSAYS:

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

Note: Volumes may be reduced proportionally without modifying results.

## CALCULATION

Calculate the result as follows:

- ✓ Refer to enclosed Standard Curves (batch specific)

or

- ✓ Plot Standard Curves on millimeter paper (Absorbances) handling as indicated in table 1.  
Abscissa: Units (IU/L)  
Ordinate: Absorbances

Transfer "Assay" absorbances on Standard Curve. Read activity (IU/L)

## REFERENCES

- (1) *TIETZ N.W. Textbook of clinical chemistry, 3<sup>rd</sup> Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 652-657*
- (2) *Clinical Guide to Laboratory Test, 4<sup>th</sup> Ed., N.W. TIETZ (2006) p. 64-67 et p.76-77.*
- (3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4<sup>th</sup> 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*