GOT / AST End-Point Colorimetric

AST reagent is for the in-vitro quantitative determination of aspartate aminotransferase activity in human serum or plasmaon manual systems.

REF:V/OT02.050	100 test	REF:V/OT02.125	250 test
REF:V/OT02.100	200 test		

CLINICAL SIGNIFICANCE [1]

The AST is a cellular enzyme, is found in highest concentration in heart muscle, the cells of the liver, the cells of the skeletal muscle and in smaller amounts in other weaves.
Although an elevated level of AST in the serum is not specific of the hepatic disease, is used mainly to diagnostic and to verify the course of this disease with other enzymes like ALT and ALP.
Also it is used to control the patients after myocardial infarction, in skeletal muscle disease and other.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

METHOD PRINCIPLE

Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from L-aspartate to 2-oxoglutarate forming L-glutamate and oxaloacetate.

L-Aspartate + 2-Oxoglutarate $\overset{\text{AST}}{\rightleftharpoons}$ **L-Glutamate + Oxaloacetate**

The AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

REAGENT COMPOSITION

R1:Buffer enzyme	
Phosphate buffer	100 mmol/L
L- aspartate	100 mmol/L
2-Oxoglutarate	5 mmol/L
NaoH	140 mmol/L
Sodium Azide	12 mmol/L
R2: Substrate	
2,4-dinitrophenyl-hydrazine	2 mmol/L
HCL	8.4 %

PRECAUTIONS AND WARNING

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie** GOT reagent material safetydata sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie AST reagents contain sodium azide which may react with copperor lead plumbing.
All reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2-8ºC.

Deterioration

The **Lab.Vie** AST reagent normally clear. Do not use the **Lab.Vie** AST regents if precipitate forms.

SPECIMEN COLLECTION AND PRESERVATION [1]

Serum or Plasma

Use only non haemolyzed serum. The only acceptable anticoagulants are heparin and EDTA. The biological half-life of AST in serum is 17 hours.
Stability: 1 day at 15-25 ºC , 7 days at 4-8 ºC , 12 weeks at -20 ºC.

SYSTEM PARAMETERS

Wavelength	546 nm (530 -550 nm)
Optical path	1Cm
Assay type	End point
Direction	Increase
Sample Reagent Ratio	1:60
Temperature	37ºc to 20-25ºc
Equilibration time	60 Sec
Zero adjustment	Reagent or Sample blank
Sensitivity	7 U/L
Linearity	89 U/L

ASSAY PROCEDURE

1- Measurement against Reagent

	Reagent Blank	*Sample*
R1	250 µl	250µl
Distilled water	50µl	
Specimen		50µl
Mix and incubate for exactly 30 minutes at 37 ºc		
R2	250 µl	250µl
Mix and incubate for exactly 20 minutes at 20-25 ºc		
Sodium hydroxide	2.5 ml	2.5 ml

Mix, and incubate for 5 minutes at 20-25 ºc then measure absorbance of specimen against reagent blank at 546 nm.

2- Measurement against Sample Blank

	Sample Blank	*Sample*
R1	250 µl	250µl
Specimen		50µl
Mix and incubate for exactly 30 minutes at 37 ºc		
R2	250 µl	250µl
Specimen	50µl	
Mix and incubate for exactly 20 minutes at 20-25 ºc		
Sodium hydroxide	2.5 ml	2.5 ml

Mix, and incubate for 5 minutes at 20-25 ºc then measure absorbance of specimen against sample blank at 546 nm.

CALCULATION

Absorbance	U/L	Absorbance	U/L
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie** technical support.

Sensitivity
When run as recommended, the minimum detection limit of this assay is 7.0 U/L.

Linearity
The reaction is linear up to AST concentration of 89 U/L; specimens showing higher concentration should be diluted 1+9 with physiological saline and repeat the assay (result×10).

INTERFERING SUBSTANCES [5]

Hemolysis
Positive interference because AST released from erythrocytes
Icterus
No significant interference.
Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.
Note
High concentration of aldehydes, ketones, or oxo-acids in some sera may cause false high transaminases levels. Measurement aganist a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

EXPECTED VALUES [2,5]

Up to 12 U/L

DYNAMIC RANGE

7 – 89 U/L

REFERENCES

1. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
2. Clinical guide to laboratory test4th ed.N.W.TIETZ(2006)p-154-159
3. Reitman S and Frankel S.Am .J.Clin.Path, 1975 ;28;65.
4. Henry RJ et al. Am J Clin Path 1960 :34:381.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		
	Expiration Date		
	Manufactured by		