ASPARTATE AMINOTRANSFERASE (AST)

OSR6109	**4 x 25 mL**	**R1**
	4 x 25 mL	**R2**
OSR6209	**4 x 50 mL**	**R1**
	4 x 50 mL	**R2**
OSR6509*	**4 x 104 mL**	**R1**
	4 x 104 mL	**R2**

Intended Use
System reagent for the quantitative determination of Aspartate Aminotransferase (EC 2.6.1.1) activity in human serum on Beckman Coulter AU analyzers.
*Aspartate Aminotransferase (AST) reagent OSR6509 for use on the AU2700/5400/680 system only.

Summary
Serum GOT (glutamic oxalacetic transaminase) is an alternate name for this enzyme which is internationally known as AST (aspartate aminotransferase) by the International Federation of Clinical Chemistry (IFCC) standards.[1]

Serum AST is one of a group of enzymes which catalyzes the interconversion of amino acids and keto acids by transfer of amino groups. Transaminases are widely distributed in body tissues with significant amounts found in the heart and liver.[2] Lesser amounts are also found in skeletal muscles, kidneys, pancreas, spleen, lungs, and brain. Injury to these tissues result in the release of the AST enzyme to general circulation.

Following a myocardial infarction, AST in serum begins to increase within 6 to 8 hours of onset of pain, reaching a peak within 18 to 24 hours and falling to normal by the fourth or fifth day. Serum values may increase to 15 to 20 times normal levels and the increase is roughly proportional to the degree of tissue damage.[3]

Methodology
This AST procedure utilizes a modification of the methodology recommended by the IFCC.[4] In this method, aspartate aminotransferase (AST) catalyzes the transamination of aspartate and α-oxoglutarate, forming L-glutamate and oxalacetate. The oxalacetate is then reduced to L-malate by malate dehydrogenase, while NADH is simultaneously converted to NAD^+.The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the AST activity in the sample.

$$\text{L-Aspartate} + \alpha\text{-Oxoglutarate} \xrightarrow{\text{AST}} \text{L-Glutamate} + \text{Oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-Malate} + NAD^+$$

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Tris buffer, pH 7.65 (37ºC)	80	mmol/L
LDH	≥ 0.9	kU/L
L- Aspartate	240	mmol/L
α-Oxoglutarate	12	mmol/L
NADH	0.20	mmol/L
MDH	≥ 0.6	kU/L

Also contains preservative.

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of Reagents
The AST Reagents are ready for use. No preparation is required.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation
Serum or heparinized plasma free from hemolysis is the recommended specimen. The concentration of AST in red cells is roughly 15 times that of normal serum; therefore, hemolysis should be avoided.[3]

Sample Storage and Stability
AST in serum is stable for 1 day when stored at 15 - 25°C or for four weeks when stored at 2 - 8°C and 1 year or more at ≤ -20°C.[5]

Aspartate Aminotransferase (AST)

Interfering Substances
Results of laboratory studies[6] show that the following substances interfere with AST determinations:

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Lipemia: No significant interference up to 300 mg/dL Intralipid*
Pyruvate: No significant interference up to 1 mmol/L Pyruvate

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[7] for a compilation of reported interferences with this test.

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
AST Reagent

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration of this AST procedure on the AU400/400^e and AU600/640/640^e is based upon the theoretical extinction coefficient for NADH, which has a molar absorptivity of 4960 at 340/380 nm. On the AU2700/5400/680/480 it is based on experimental determination of the molar absorptivity at 340/660nm.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in U/L at 37°C.

Dynamic Range

The AST procedure is linear from 3 to 1000 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Expected Values

Adults:[5] 13 - 39 U/L

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the AST Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ,

Precision[9]
Estimates of precision, based on CLSI recommendations[8], are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of control sera were carried out and data reduced following CLSI guidelines above:

N = 60	Within run		Total	
Mean, U/L	SD	CV%	SD	CV%
21	0.7	3.5	0.8	3.7
195	0.8	0.4	1.8	0.9

Method Comparison[9]
Patient samples were used to compare this AST Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	AU600
Slope	1.029
Intercept	0.6
Correlation Coeff. (r)	0.9998
No. of Samples (n)	184
Range (U/L)	3-433

Sensitivity
Typical change in absorbance per minute for 1 U/L of AST is 0.19 mAbsorbance at 340/380nm and 0.22 mAbsorbance at 340/660nm.

References

1. International Federation of Clinical Chemistry. Clin Chem; 23: 887, 1977.
2. Wilkinson, J.H., The Principles and Practice of Diagnostic Enzymology, Year Book Medical, 1976.
3. Tietz, N.W. (ed), Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders, 1994.
4. Saris, N.E., Clin Chem, 24: 720 - 721, 1987.
5. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
6. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP7-P, 1986.
7. Young, D.S., Effects of Drugs on Clinical Laboratory Results, 5th Edition, AACC Press, 2000.
8. CLSI/NCCLS Evaluation Protocol, EP5-T2, 1992.
9. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA

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