BECKMAN COULTER

AU/DxC AU US

Instructions For Use

AST

ASPARTATE AMINOTRANSFERASE (AST)

REF OSR6109 4 x 25 mL R1, 4 x 25 mL R2
OSR6209 4 x 50 mL R1, 4 x 50 mL R2
OSR6609 4 x 173 mL R1, 4 x 173 mL R2

For *in vitro* diagnostic use only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Aspartate Aminotransferase (EC 2.6.1.1) activity in human serum on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

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.

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

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

SPECIMEN

SPECIMEN STORAGE AND STABILITY

AST is stable in serum/plasma for 7 days when stored at 4 - 8°C, 4 days when stored at 20 - 25°C and 3 months when stored at -20°C. [5]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum or Li/Na 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]

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

AST Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

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.	

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

AST R1	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Tris(hydroxymethyl)– aminomethane 5 - 8%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR6609 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

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.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

Calibration of this AST procedure on the AU5800/680/480, DxC 700 AU, DxC 500i and DxC 500 AU is based on experimental determination of the molar absorptivity at 340/660 nM.

The theoretical extinction coefficient for AST is 4127.

DxC 500 AU and DxC 500i CALIBRATION INFORMATION ONLY

Ensure that Manufacturer Factor A is 1.0.

Prepare 3 fresh vials of System Calibrator Cat No. 66300X and run as samples in duplicate. Examine the data for outliers which should be repeated and replaced and calculate the **Overall Mean** Value. Calculate the analyzer specific Manufacturer Factor A:

$$\textbf{Calculated Factor} = \frac{\textbf{System Calibrator REF 66300X Set Point per product Insert}}{\textbf{Overall Mean Value}}$$

Enter the **Calculated Factor** in the Manufacturer Factor A field in the General Parameters section of the Chemistry Details Screen. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

Re-establishment of the analyzer specific Manufacturer Factor A is recommended when QC results are not within the laboratories' established ranges following replacement of a critical part of the analyzer.

Reagent blank measurement is recommended when changing to a new lot of reagent.

AU480 / AU680 / DxC 700 AU / AU5800 CALIBRATION INFORMATION ONLY

Ensure that the MB Factor in the MB Type Factor field is set to the assay specific **Theoretical Extinction Coefficient.** Prepare 3 fresh vials of System Calibrator Cat No. 66300X and run as samples in duplicate. Examine the data for outliers which should be repeated and replaced and calculate the **Overall Mean** Value. Calculate the **Derived Multiplier** :

$$\textbf{Derived Multiplier} = \frac{\textbf{System Calibrator REF 66300X Set Point per product Insert}}{\textbf{Overall Mean Value}}$$

Multiply the **Theoretical Extinction Coefficient** for the assay by the **Derived Multiplier** to get the **analyzer specific MB type factor**. Enter the **analyzer specific MB type Factor** in the MB Type Factor section of the calibration specific menu.

For the AU5800, ensure the correct unit and cuvette wheel is selected. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

Re-establishment of the analyzer specific MB factor is recommended when QC results are not within the laboratories' established ranges following replacement of a critical part of the analyzer.

Reagent blank measurement is recommended when changing to a new lot of reagent.

Note for the AU5800 analyzer: the analyzer specific MB Factor is generated for each analyzer unit and cuvette wheel that the enzyme assay is used.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC 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 after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is U/L, for conversion to SI units (µkat/L) the result is divided by 60.

REPORTING RESULTS

EXPECTED RESULTS

Reference[6]

Male: <50 U/L

Female: <35 U/L

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of laboratory studies[7] 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. Further information on interfering substances is available.[8]

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The AST procedure is linear from 3 to 1,000 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.

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.

METHODS COMPARISON

Reference[9]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 700 AU
X Method	AU5800
Slope	0.988
Intercept	-0.04
Correlation Coeff. (r)	1.0000
No. of Samples (n)	129
Range (U/L)	5 - 859

PRECISION

Reference[9]

Estimates of precision, based on CLSI recommendations[10], 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 = 80	Within run		Total	
Mean, U/L	**SD**	**CV%**	**SD**	**CV%**
17.3	0.49	2.8	0.62	3.6
47.7	0.62	1.3	0.78	1.6
311.0	1.68	0.5	3.60	1.2

ADDITIONAL INFORMATION

DxC 700 AU analyzers require that each reagent application has a standard format of abbreviated Test Name. This Test Name is required to allow automated loading of the calibrator information for each application. Refer to the table below for the Test Name assigned to each application for this assay.

Test Name	Description
AST1U	AST (Serum)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

* Values set for working in U/L. To work in SI units (µkat/L) divide by 60.

The theoretical extinction coefficient is 4127

Ψ Analyzer Specific MB Factor = Theoretical Extinction Coefficient*Derived Multiplier.

AU5800 only:

MB Factor adjustment must be completed separately for each ring.

REVISION HISTORY

Add DxC 500i instrument to IFU

Preceding version revision history

Updated Specimen Section

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

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.
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4. Schumann G, Bonora R, Ceriotti F et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Part 5. Reference Procedure for the Measurement of Catalytic Concentration of Aspartate Aminotransferase. Clin Chem Lab Med 2002;40:725-733.
5. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of Anticoagulants in Diagnostic Laboratory Investigations and Stability of Blood, Plasma and Serum Samples. WHO/DIL/LAB/99.1 Rev.2:23pp.
6. Thomas L, Müller M, Schumann G et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005;29:301-08.
7. CLSI. Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition. CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
8. AACC Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs and Natural Products https://clinfx.wiley.com/aaccweb/aacc/
9. Data is on file for specific AU/DxC AU analyzers.
10. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
+(1) 800-854-3633
www.beckmancoulter.com