AST

Aspartate Aminotransferase
IFCC
RX DAYTONA PLUS

INTENDED USE

An AST test system is a device intended for the quantitative *in vitro* determination of aspartate aminotransferase (AST) activity in serum and plasma. This product is suitable for use on the RX **series** instrument RX **daytona plus**.

For prescription use only.

Cat. No.

AS 8306	R1. Buffer/Enzyme	4 x 20 ml
	R2. α-oxoglutarate/Coenzyme	4 x 7 ml

GTIN: 05055273208245

UV METHOD

This is a modification of the optimised standard method according to the recommendations of the IFCC.

CLINICAL SIGNIFICANCE[1,2,3,4]

The aminotransferases are a group of enzymes that catalyse the inter conversions of amino acids and α-oxoacids by transfer of amino groups. AST (aspartate aminotransferase or glutamate oxaloacetate transaminase) has been found in the cytoplasm and the mitochondria of cells that have been studied. In cases of mild tissue damage (e.g. liver), the predominant form of serum AST is that from the cytoplasm, with a smaller amount coming from the mitochondria. Severe tissue damage will result in more mitochondrial enzyme being released. Elevated levels of AST can signal myocardial infarction, hepatic disease, muscular dystrophy and organ damage.

Although heart muscle is found to have the most activity of the enzyme, significant activity has also been seen in the brain, liver, gastric mucosa, adipose tissue and kidneys of humans.

The IFCC has now recommended (1980)[1] standardised procedures for AST determinations including:-

1. Optimization of substrate concentrations.
2. Employment of Tris buffers (instead of phosphate, which has been shown to inhibit recombination of the apoenzyme with pyridoxal phosphate).
3. Pre-incubation of combined buffer and serum to allow side reactions with NADH to occur.
4. Substrate start (α-oxoglutarate).
5. Optional pyridoxal phosphate activation.
   This is an optimised standard method according to the recommendations of the IFCC.

PRINCIPLE

α-oxoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction utilises the oxaloacetate for a kinetic determination of NADH consumption.

$$\text{I-oxoglutarate + L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate + oxaloacetate}$$

$$\text{oxaloacetate + NADH + H}^+ \xrightarrow{\text{MDH}} \text{L-malate + NAD}^+$$

SPECIMEN COLLECTION AND PREPARATION [5]

Serum: - Use serum free from haemolysis.

Plasma: Lithium heparin can be used as the anticoagulant. Plasma should be separated from cells within one hour after collection.
Specimens should be refrigerated if not used immediately.
Specimens stored longer than 3 days should be frozen at –20°C.

REAGENT COMPOSITION

Contents		Concentration in the Test
R1.	**Buffer/Enzyme**	
	Tris buffer	80 mmol/l, pH 7.5
	L-Aspartic acid	240 mmol/l
	MDH	≥ 0.42 U/ml
	LD	≥ 0.60 U/ml
R2.	**α-oxoglutarate/Coenzyme**	
	α-oxoglutarate	12 mmol/l
	NADH	0.24 mmol/l

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1 and R2 contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Safety Data Sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENT

R1. Buffer/Enzyme
Contents ready for use as supplied. Stable up to the expiry date when stored at +2 to +8°C.

R2. α-oxoglutarate/Coenzyme
Contents ready for use as supplied. Stable up to the expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED
Buffer/Enzyme
α-oxoglutarate/Coenzyme

MATERIALS REQUIRED BUT NOT PROVIDED
Randox Assayed Human Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)
RX **series** Saline (Cat. No. SA 8396)

PROCEDURE NOTES
To avoid the potential for reagent carryover, it is recommended that the testing order of the reagents is confirmed. Please consult the reagent carryover document available on www.randox.com under support and documentation - Reagent product inserts or by contacting Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

The Chemistry parameters for Randox Dedicated RX **daytona plus** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

RX DAYTONA PLUS CALIBRATION
The use of Saline and Randox Calibration Serum Level 3 is recommended for calibration.

STANDARDISATION
Randox Calibration Serum Level 3 is traceable to AST reference material JSCC TS01.

QUALITY CONTROL
Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water. Contaminants, i.e. bacterial growth, may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

NORMAL VALUES[6,7]

	+37°C
Men	up to 35 U/l
Women	up to 31 U/l

TEMPERATURE CONVERSION FACTORS [8]
+30 / 25°C: 1.41 ± 0.07
+37 / 25°C: 2.21 ± 0.15

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance characteristics were obtained using a RX **daytona plus** analyser at +37°C.

SPECIFICITY/INTERFERENCE[9,10]
Gross haemolysis will produce falsely elevated test results. The effects of various drugs on AST activity should be taken into consideration in the case of patients receiving large doses of drugs.

The analytes below were tested up to the levels indicated at AST concentrations of 35.0 U/l and were found not to interfere:

	35.0 U/l
Haemoglobin	Interferes
Total Bilirubin	60 mg/dl
Conjugate Bilirubin	60 mg/dl
Triglycerides	500 mg/dl
Intralipid®	500 mg/dl

A list of substances and conditions known to effect AST activity *in vivo* is given by both Young *et al* and Friedman *et al.* No representation is made by Randox Laboratories Ltd regarding the completeness of these lists and the accuracy of the information contained therein.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [11]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

LINEARITY
Linearity data demonstrates the reportable range for AST is 5 to 1116 U/l on the RX **daytona plus** analyser.

SENSITIVITY
The Limit of Detection (L.o.D), the Limit of Blank (L.o.B) and Limit of Quantitation (L.o.Q) were determined consistent with CLSI guidelines EP17-A. L.o.D is the smallest concentration that can be detected to determine the presence or absence of AST. L.o.B is the highest concentration that is likely to be observed in a blank sample. L.o.Q is the lowest amount of analyte in a sample that can be quantitatively determined with stated acceptable precision and trueness, under stated experimental conditions.

The L.o.B for AST on the RX **daytona plus** is 0.50 U/l.
The L.o.D for AST on the RX **daytona plus** is 1.372 U/l.
The L.o.Q for AST on the RX **daytona plus** is 5 U/l.

PRECISION

Within run precision

	Level 1	Level 2	Level 3
Mean (U/l)	18.8	37.8	162
SD	0.428	1.14	2.23
CV(%)	2.27	3.00	1.38
n	80	80	80

Total precision

	Level 1	Level 2	Level 3
Mean (U/l)	18.8	37.8	162
SD	1.08	1.29	2.69
CV(%)	5.75	3.42	1.66
n	80	80	80

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.03X + 2.33
and a correlation coefficient of r = 0.999

92 patient samples were analysed spanning the range 5.0 to 817 U/l.

REFERENCES

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9. Young D S, *et al*: Clin Chem 1975, 21; No5.
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The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP

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