AST

Aspartate Aminotransferase
IFCC
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Aspartate Aminotransferase (AST) in serum and plasma. This product is suitable for manual use and on the Rx Monza analyser.

Cat. No.			
AS 1202	R1a.	Buffer/Substrate	1 x 70 ml
20 x 2 ml	R1b.	Enzyme/Coenzyme/ α-oxoglutarate	20 x 2 ml
GTIN:		05055273200416	
AS 1204	R1a.	Buffer/Substrate	1 x 105 ml
10 x 10 ml	R1b.	Enzyme/Coenzyme/ α-oxoglutarate	10 x 10 ml
GTIN:		05055273200423	
AS 2359	R1a.	Buffer/Substrate	5 x 100 ml
5 x 100 ml	R1b.	Enzyme/Coenzyme/ α-oxoglutarate	5 x 100 ml
GTIN:		05055273200454	

UV METHOD

This is an optimised standard method according to the concentrations recommended by 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) 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.

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

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

SPECIMEN COLLECTION AND PREPARATION [5]

Serum: - Use serum free from haemolysis.
Plasma: - EDTA or 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		Concentrations in the Test
R1a.	**Buffer/Substrate**	
	Tris buffer	80 mmol/l, pH 7.5
	L-aspartate	240 mmol/l
R1b.	**Enzyme/Coenzyme/α-oxoglutarate**	
	α-oxoglutarate	12 mmol/l
	MDH	≥420 U/l
	LD	≥600 U/l
	NADH	0.18 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.

Solution R1a contains 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.

Health and Safety data sheets available on request.

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

STABILITY AND PREPARATION OF REAGENTS

R1a. Buffer/Substrate
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R1b. Enzyme/Coenzyme/α-oxoglutarate
Reconstitute one vial of Enzyme/Coenzyme/ α-oxoglutarate R1b with the appropriate volume of Buffer/Substrate R1a:
2 ml for the **20 x 2 ml** kit (AS 1202)
10 ml for the **10 x 10 ml** kit (AS 1204)
Stable for 14 days at +2 to +8°C or 24 hours at +15 to +25°C.
Cat. AS 2359 5 x 100 ml
Reconstitute one vial of Enzyme/Coenzyme/ α-oxoglutarate R1b with a portion of Buffer/Substrate R1a and then transfer the entire contents to bottle R1a rinsing bottle R1b several times. Stable for 14 days at +2 to +8°C or 24 hours at +15 to +25°C.

MATERIALS PROVIDED

Buffer/Substrate
Enzyme/Coenzyme/α-oxoglutarate

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed 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 3854)

PROCEDURE

Aspirate fresh ddH_2O and perform a new Gain Calibration in flow cell mode. Select AST in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:	
Sample	0.05 ml
Reagent	0.5 ml

Mix and aspirate into the Rx Monza.

CALIBRATION FOR RX MONZA

The use of Saline and Randox Calibration Serum Level 3 is recommended for calibration. Calibration is recommended with change of reagent lot or as indicated by quality control procedures.

FOR MANUAL USE

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm light path
Temperature:	25/30/37°C
Measurement:	against air

Pipette into cuvette:

	Macro	Micro
Sample	0.2 ml	0.1 ml
Enzyme/Coenzyme/ α-oxoglutarate R1	2.0 ml	1.0 ml

Mix, read initial absorbance after 1 minute. Read again after 1, 2 and 3 minutes. Note: If the absorbance change per minute is between:

0.11 and 0.16 at 340/Hg 334 nm
0.06 and 0.08 at Hg 365 nm

use only the values for the first 2 minutes for the calculation.

MANUAL CALCULATION

To calculate the AST activity, use the following formulae:

U/l = 1746 x Δ A 340 nm/min
U/l = 1780 x Δ A Hg 334 nm/min
U/l = 3235 x Δ A Hg 365 nm/min

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.

SPECIFICITY/INTERFERENCE [6,7]

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 following levels and were found not to interfere:

Haemoglobin	250 mg/dl
Free Bilirubin	25 mg/dl
Conjugate Bilirubin	25 mg/dl
Triglycerides	1000 mg/dl
Intralipid®	200 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.

NORMAL VALUES IN SERUM [8,9]

	+25°C	+30°C	+37°C
Men	up to 18 U/l	up to 25 U/l	up to 37 U/l
Women	up to 15 U/l	up to 21 U/l	up to 31 U/l

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 data were obtained using an Rx Monza analyser running at +37°C.

LINEARITY

This method is linear up to 562 U/l. If the sample concentration exceeds this value, dilute the sample 1+9 with 0.9% NaCl solution and re-assay. Multiply the result by 10.

SENSITIVITY

The minimum detectable concentration of AST with an acceptable level of precision was determined as 9.3 U/l.

PRECISION

Intra Assay

	Level 2	Level 3
Mean (U/l)	35.6	153
SD	1.66	1.47
CV (%)	4.65	0.96
n	20	20

Inter Assay

	Level 2	Level 3
Mean (U/l)	35.6	153
SD	1.77	7.10
CV (%)	4.96	4.63
n	20	20

CORRELATION

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

Y = 1.07X + 4.9
and a correlation coefficient of r = 0.9975

43 patient samples were analysed spanning the range 28 to 559 U/l.

REFERENCES

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