COD 11830	COD 11531	COD 11567	COD 11561
1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
STORE AT 2-8ºC			
Reagents for measurement of AST/GOT concentration Only for *in vitro* use in the clinical laboratory			

ASPARTATE AMINOTRANSFERASE (AST/GOT)

BioSystems

ASPARTATE AMINOTRANSFERASE (AST/GOT) IFCC

PRINCIPLE OF THE METHOD

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction[1,2,3].

$$\text{Aspartate} + 2\text{-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + \text{Glutamate}$$

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

CONTENTS

	COD 11830	COD 11531	COD 11567	COD 11561
A. Reagent	1 x 40 mL	1 x 160 mL	1 x 400 mL	1 x 800 mL
B. Reagent	1 x 10 mL	1 x 40 mL	1 x 100 mL	1 x 200 mL

COMPOSITION

A. Reagent: Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, pH 7.8.
WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention.

B. Reagent: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L
WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank lower than 1.400 at 340 nm (1 cm cuvette).

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

AUXILIARY REAGENTS

C. Reagent (cod 11666): Pyridoxal phosphate AST 10 mmol/L. 5 mL.

REAGENT PREPARATION

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B (Note 1). Stable for 1 month at 2-8ºC.

Working Reagent with Pyridoxal Phosphate (Note 2): Mix as follows: 10 mL of Working Reagent + 0.1 mL of Reagent C (cod 11666). Stable for 6 days at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 340 nm.
– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Aspartate aminotransferase in serum and plasma is stable for 7 days at 2-8ºC. Use heparin as anticoagulant[7].

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 3)

Reaction temperature	37ºC
Working Reagent	1.0 mL
Sample	50 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 1 minute (Note 1), record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The AST/GOT concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = \text{U/L}$$

The molar absorbance (ε) of NADH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05 at 37ºC, the sample volume (Vs) is 0.05 at 37ºC and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

	37ºC
ΔA/min	x 3333 = U/L x 55.55 = µkat/L

REFERENCE VALUES

Reaction temperature	37ºC
Without pyr-P, up to[4]	40 U/L = 0.67 µkat/L
With pyr-P, up to[2]	50 U/L = 0.83 µkat/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.67 U/L = 0.028 µkat/L
– Linearity limit: 800 U/L = 13.3 µkat/L. For higher values dilute sample 1/ 10 with distilled water and repeat measurement.
– Repeatibility (within run):

Mean Concentration	CV	n
38 U/L = 0.63 µkat/L	1.4 %	20
119 U/L = 1.98 µkat/L	1.5 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
38 U/L = 0.63 µkat/L	5.9 %	25
119 U/L = 1.98 µkat/L	3.8 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
– Interferences: Lipemia (triglycerides 2 g/L) interfere. Bilirubin (20 mg/dL) and hemolysis (hemoglobin 50 mg/dL) do not interfere. Other drugs and substances may interfere[5].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

The aminotransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. AST is found in highest concentration in the liver and heart muscle but it is also abundant in skeletal muscle, kidney and pancreas.

The serum concentration of AST is elevated in hepatitis and other forms of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastasic carcinoma of the liver, delirium tremens, and after administration of various drugs[4,6].

Serum AST concentration is also elevated after myocardial infarction, in sketetal muscle disease (as progressive muscular distrophy), in acute pancreatitis or hemolytic disease and other[4,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. The initial absorbance of the reaction mixture may be out of range in some photometers with a low maximum absorbance reading. For these photometers it is recommended to prepare the Working Reagent by mixing in the proportion: 5 mL Reagent A + 1 mL Reagent B.
2. The IFCC recommended method specifies the addition of pyridoxal phosphate. The delay time before measurements should then be increased to 2 minutes.
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

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