NS Bio-Tec

ASAT/AST/GOT) - Kinetic

GOT-MK-1010

10X10 ML

INTENDED FOR USE

For the determination of AST/GOT in serum

Present Kinetic method for determination of aspartate amino transferase without perodoxal-phosphate is optimized in accordance with I.F.C.C. recommendations.

Aspartate amino transferase (AST/GOT) catalyzes the following reaction:

AST/GOT

 α -ketoglutarate + L-aspartate

 \Leftrightarrow

L-glutamate + oxaloacetate

By the coupled reaction of Malate -hydrogenase (MDH) and the relative co enzyme (NADH), oxaloacetate is reduced to Malate with the co enzyme oxidation

MDH

oxaloacetate + NADH + H+

L-Malate + $NAD^+ + H_2O$ \Leftrightarrow

The reduced co enzyme consumption, observed as decrease of the per time extinction, is proportional to AST/GOT activity in the sample.

SPECIMEN COLLECTION:

Non hemolized serum or plasma.

Common anticoagulants can be used.

(Heparin, EDTA, oxalates, and fluorides).

REAGENTS COMPOSITION:

R1	Buffer/Substrate (Liquid) Phosphate buffer L-Aspartate	80 mmol/l 200 mmol/l
R2	Enzymes/coenzyme (liquid) LDH MDH NADH α - Oxoglutarate	≥ 1.2 U/ml ≥ 0.6 U/ml ≥ 0.18 mmol/l 12 mmol/l

PACKAGE: Collection & Storage.

Store at +2-8°C.

Stable until the expiration date reported upon the package.

After the unsealing and the taking of the reagent, it is advised to close up the bottle immediately in order to avoid evaporation, direct light exposure and bacterial contamination.

PRECAUTIONS & WARNING:

Avoid pipette by mouth.

The preparation, according to current regulation, is classified as not dangerous. The total concentration of non active components (preservatives, detergents, and stabilizers) is below the minimum required for citation. Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis

REAGENT PREPARATION & STABILITY:

Liquid reagents must be at room temperature (+15-25°C) before using. Reagent (R1) is limpid/ colorless; Reagent (R2) is from colorless to pale

Add 4 parts of Reagent (R1) to 1 part of Reagent (R2).

Reagent (R1+R2) is reported stable up to 4 days at room temperature and 4 weeks if stored in refrigerator.

REQUIRED MATERIALS NOT PROVIDED:

General Laboratory Equipment and instrumentations

PROCEDURE:

Wavelength 340,334 or 365 nm

Optical path 1 cm

Incubation temperature 25,30 or 37°C Zero adjustment Against air

Pipette into cuvette

Macro	Semi-micro
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Specimen	200 μ1	100 μ1
Working solution	2.0 ml	1.0 ml

Mix, read initial absorbance after 60 sec. and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (ΔA/min).

CALCULATION:

To calculate the AST /GOT activity use the following formula

 $U/l = 1780 \text{ x } \Delta A 334 \text{ nm / min}$

 $U/l = 1746 \text{ x } \Delta A 340 \text{ nm / min}$

 $U/l = 3235 \text{ x} \Delta A 365 \text{ nm} / \text{min}$

EXPECTED VALUES:

	25°C	30°C	37°C
Female	Up to 15 U/l	Up to 21 U/l	Up to 31 U/l
Male	Up to 18 U/l	Up to 25 U/l	Up to 37 U/l

The above mentioned values are to be considered as a reference. It is strongly recommended that each laboratory establish its own normal range.

WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

OUALITY CONTROL:

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology.

REFERENCES:

- 1. Tietz, N.W. (ed) Fundamentals of Clinical Chemistry W.B. Saunders Co., Philadelphia, 1976.
- 2. Henry, R.J. Clinical Chemistry, Principles and Technics. Harper and Row Publishers. New York, 1964.

3. Provisional Recommendations on IFCC methods for the measurement of catalytic concentrations

of enzymes. Clin chem. 23(887), 1977.

PERFORMANCE:

MEASURE INTERVAL :	0-500 U/L
MEASURABLE LIMIT:	3 U/L
SENSITIVITY:	1 U/L= 0.0017 ΔE/min.

PRECISION WITHIN SERIES: n=20

HIGH LEVEL M= 156U/L	C.V.=2.1%
PRECISION AMONG SERIES: n=20	C. V2.170

LOW LEVEL	M= 31U/L	C.V.=3.1%	
HIGH LEVEL	M= 160U/L	C.V.=3.8%	
CORRELATION	r = 0.998	n=50	
LIN. REGRESSION	y = 0.96x + 1.33	n=50	

INTERFERENCE:

Interferences are	negligible up to:		
Bilirubin	18 mg/dL	Triglycerides	500 mg/dL
Hemoglobin	300 mg/dL	Glucose	500 mg/dL

For concentration higher than 500 U/L for turbid and\or icteric specimens, repeat the measure on a sample diluted 1:10 with physiological saline and multiply the results by 10.

due to AST presence in erythrocytes.

Individuals lacking B6 vitamin may present low AST level, probably due to pirid-oxalic-phosphate absence.

For a through evaluation of the interfering substances, consult: Young, D.S., et al., Clin. Chem. 21:1D (1975).



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