AST (GOT)

Aspartate aminotransferase
E.C.2.6.1.1.
Colorimetric method

Cat.No. 101-0025 *Size 200 tests*

PRINCIPLE:

The enzyme AST catalyzes the following reaction:

$$\text{L - aspartate} + \text{2 - oxoglutarate} \xrightarrow{\text{AST}} \text{oxalacetae} + \text{L} - \text{glutamate}$$

The oxalacetate in reaction with 2,4-dinitro- phenylhydrazine forms oxalacetate hydrazones which are brown in alkaline medium. The product is determined photometrically at 505 nm.

SAMPLE:

Serum (nonhemolyzed).

REAGENTS:

1. Buffer-substrate (1 x 100 ml)
   Triethanolamine-EDTA buffer, pH 7.5 — 50 mmol/L
   L-aspartate — 200 mmol/L
   2-oxoglutarate — 2 mmol/L
2. Color reagent (1 x 100 ml)
   2,4-dinitrophenylhydrazine (DNPH) — 1 mmol/L
3. Standard (1 x 10 ml)
   Sodium pyruvate — 2 mmol/L
4. Additional reagent
   NaOH (Cat. No. 101-0023) — 0.4 mol/L

All reagents to be used undiluted.
Reagents are stable up to the expiry date when stored at +2 °C to +8 °C.

PROCEDURE:

Wavelength:	505 nm (490-520)
Cuvette:	1 cm light path
Temperature:	37 °C
Color stability:	60 min.
Zero:	Reagent blank

Pipette into test tubes:	Sample	Reagent blank
Buffer-substrate	0.5 ml	0.5 ml
Sample	0.1 ml	-
Distilled water	-	0.1 ml
Mix and incubate for exactly 30 min. at 37 °C.		
DNPH	0.5 ml	0.5 ml
Mix well and let stand for exactly 20 min. at 20 to 25 °C.		
NaOH	5.0 ml	5.0 ml
Mix and after 5 minutes read the absorbance of sample against the reagent blank.		

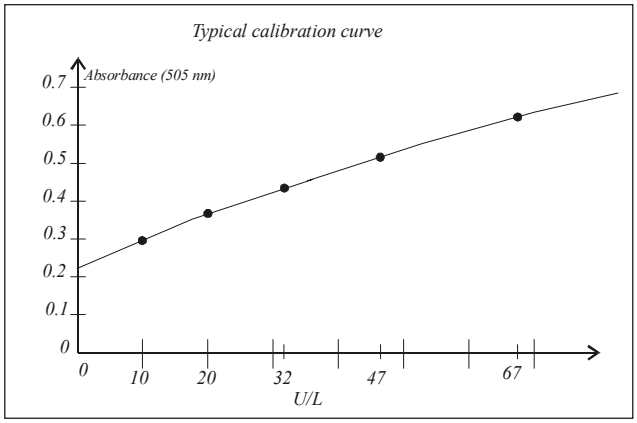
CALCULATION:

Using the absorbance values of the samples, read off the enzyme activity in U/L from calibration curve.

PREPARATION OF CALIBRATION CURVE:

Pipette into tubes:	Test tube no.						
	Blank	1	2	3	4	5	6
Redistilled water	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Buffer-substrat	1.00	0.95	0.90	0.85	0.80	0.75	0.70
Standard	-	0.05	0.10	0.15	0.20	0.25	0.30
DNPH	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mix and let stand for 20 minutes at 20°C to 25°C							
0.4 mol/L NaOH	10	10	10	10	10	10	10
Mix and after 5 minutes measure the absorbance of each solution in test tubes 1 to 8 against the reagent blank at 505 nm (or green filter).							
AST U/L	0	10	20	32	47	67	93

Plot (milimeter paper) the absorbance values determined on the ordinate against the U/L values (from the table) on the abscissa and use these points to draw a calibration curve.



EXPECTED VALUES:

AST up to 19 U/L (317 nkat/L)

LINEARITY:

up to 80 U/L (1334 nkat/L)

QUALITY CONTROL:

All commercial control sera with established values for this method.

NOTE:

1. If the activity is higher than 80 U/L, dilute sample 1:5 with saline and multiply the result by 5.
2. Buffer-substrate and standard contain sodium azide. Avoid ingestion or contact with skin or mucous membranes.

REFERENCES:

1. Reitman,S., and Frankel, S.,Amer.J.Clin.Path., 1957;28:56
2. Bergmeyer, H.U., Clin. Chem. 1972;18:1305