

# AST/GOT (C)

## **Colorimetric Method**



## PRODUCT CODE CZ006

#### INTENDED USE

This reagent is intended for *in vitro* quantitative determination of AST/GOT in serum.

#### METHOD

Colorimetric, Reitman-Frankel Method

#### **CLINICAL SIGNIFICANCE**

The AST a cellular enzyme, it is present in most of the tissues. Especially in cardiac muscle, liver cells, skeletal muscle & kidneys. Injury to these tissues results in the release of the enzyme in blood stream. Increased levels are found in myocardial infarction. The duration & extent of increase is related to the infract. GOT determination is of considerable value to differentiate myocardial infraction from other cardiac disorders. Increased levels are also found in various types of liver disease, skeletal muscle trauma & in renal diseases. Decreased levels may be found in pregnancy, Beriberi & Diabetic ketoacidosis.

#### **PRINCIPLE**

AST determination is based on the following reaction:

AST/GOT

L-Aspartate + 2-Oxoglutarate -----→Oxaloacetate +L-Glutamate

Oxaloacetate formed reacts with 2-4-dinitrophenyl hydrazine to yield a colored hydrazone that can be measured at  $505\ \mathrm{nm}$ .

#### REAGENT COMPOSITION

#### **REAGENT 1 (SUBSTRATE)**

Phosphate buffer pH 7.4 100 mmol/L L-Aspartate 200 mmol/L 2-Oxoglutarate 4 mmol/L

## REAGENT 2 (COLORREAGENT)

2-4-dinitrophenyl 1 mmol/L

hydrazine

**STANDARD** 

Pyruvic Standard 1.2 mmol/L

Additional Reagent, but not provided

Sodium hydroxide 0.4 mol/L

#### REAGENT PREPARATION

Reagents and standard are ready to use.

#### REAGENT STORAGE AND STABILITY

The reagents are stable, if protected from light, up to the stated expiry date when stored at 2 -  $8^{\circ}$  C.

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Muslim Al Attar Street, P.O.Box:1235,

#### **SPECIMEN**

Serum, free of hemolysis.

## PRECAUTION

- 1- To avoid contamination, use clean laboratory wares.
- 2- Avoid direct exposure of reagent to light.

ASSAY

 $\begin{array}{lll} Wave length & : & 505 \ nm \ (490\text{-}520 \ nm) \\ Cuvette & : & 1 \ cm \ light \ path \end{array}$ 

Temperature : 37°C

PRO	M	$^{\circ}$ F	D	IJR	$\mathbf{E}$

	GOT
Reagent-1, (Substrate)	1 mL
Incubate for 5 minutes at 37°C	
Serum	0.2 mL
Mix and incubate at 37°C for 60 minutes	
Reagent-2 (Color)	1 mL
Mix and let 20 minutes at room temperature	1
NaOH 0.4N	10 mL
Mix, wait for 5 minutes. Measure under condition	ons identical to
those used for the standard curve.	
The color intensity stable for one hour	

#### CALCULATION

From absorbencies, read unit of GOT from corresponding curves.

#### CALIBRATION (mL)

CALIBRATION (IIIL)						
Pipette into cuvettes	1	2	3	4	5	6
Distilled Water	0.2	0.2	0.2	0.2	0.2	0.2
Reagent 1 Substrate	1.0	0.9	0.8	0.7	0.6	0.5
Pyruvic standard		0.1	0.2	0.3	0.4	0.5
Reagent 2 Color	1.0	1.0	1.0	1.0	1.0	1.0
Mix, let stand for 20 minutes at room temperature						
NaOH 0.4 N	10	10	10	10	10	10
Mix, wait for 5 minutes, read absorbance of all tubes.						
Plot the standard curve of the absorbance found VS the						
corresponding unit, on a graph paper, according to the following						
concentrations						
GOT U/mL	0	22	55	95	150	215

#### LINEARITY

When GOT exceeds 165 U/mL, re-measure diluting the sample 1:10 in 9 g/L Sodium chloride.

## NORMAL RANGE

GOT/AST: <40 units/mL

## QUALITY CONTROL

All control sera with values determined by this method can be used.

## SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
Ω	Expiry Date	VOL	Volume
*	Storage Condition	LOT	Lot Number
[]i	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date	•••	Manufacturer
Σ	Number of Tests	2	For Single Use Only
EC REP	EC Representative	Œ	European conformity

#### BIBILOGRAPHY

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- 2- Tietz, NW.,Fund of Clinical Chem., 446 (1970)
- 3- Schmidt, E., Enzymology Biol.Clin., 3,1 (1963)





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Amman-11953,Jordan Tel:+962 64892525, Fax: +962 64892526, www.bioresearch.com.jo