

AST/GOT (C) **COLORIMETRIC METHOD**



REF NO DESCRIPTION

CZ904C AST/GOT (COLORIMETRIC))

PAKAGE SIZE

| CZ 904C-1 | 2 X 50 ML |
|-----------|------------|
| CZ 904C-2 | 2 X 100 ML |

INTENDED USE

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

INTENDED USER: Professional Use Only

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 nm.

REAGENT COMPOSITION **REAGENT 1 (SUBSTRATE)**

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

REAGENT 2 (COLOR REAGENT)

2-4-dinitrophenyl hydrazine 1 mmol/L

STANDARD

1.2 mmol/L Pyruvic Standard

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° C.

SPECIMEN

Serum, free of hemolysis.

PRECAUTION

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

ASSAY

Wavelength 505 nm(490-520 nm) Cuvette 1 cm light path

Temperature 37°C

Against distilled water Measurement

PROCEDURE

| | 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 | I | | | | |
| Reagent-2 (Color) | 1 mL | | | | |
| Mix and let 20 minutes at room temperature | 1 | | | | |
| NaOH 0.4N | 10 mL | | | | |
| , wait for 5 minutes. Measure under conditions 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)

| 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 minu | Mix, let stand for 20 minutes at room temperature | | | | | | | |
| NaOH 0.4 N | 10 | 10 | 10 | 10 | 10 | 10 | | |
| Mix, wait for 5 minutes, re | e 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.

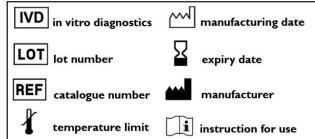
NORMAL RANGE

GOT/AST: <40 units/mL

QUALITY CONTROL

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

SYMBOL ON LABELS



BIBILOGRAPHY

- Reitman S., Frankel S., Am. Clin. Pathol., 28,56 (1957) 1-
- Tietz, NW., Fund of Clinical Chem., 446 (1970) 2-
- Schmidt, E., Enzymology Biol.Clin., 3,1 (1963)



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