

# ALT/GPT (C) COLORIMETRIC METHOD



**REF NO** 

DESCRIPTION

ALT/GPT (COLORIMETRIC)) CZ902C

#### PAKAGE SIZE

| CZ 902C-1 | 2 X 50 ML  |  |  |
|-----------|------------|--|--|
| CZ 902C-2 | 2 X 100 ML |  |  |

#### INTENDED USE

This reagent is intended for in vitro quantitative determination of ALT/GPT in serum.

INTENDED USER: Professional Use Only

COLORIMETRIC, REITMAN-FRANKEL METHOD

#### CLINICAL SIGNIFICANCE

ALT is present in high concentrations in the liver and to a lesser extent in kidney, heart and skeletal muscle, pancreas, spleen and lung. Increased levels of ALT however are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. Characteristically ALT is generally higher than AST in acute viral or toxic hepatitis, whereas for most patients with chronic hepatic disease, ALT levels are generally lower than AST levels. Elevated ALT levels have also been found in extensive trauma and muscle disease. circulatory failure with shock, hypoxia, myocardial infarction and haemolytic disease.

#### PRINCIPLE

ALT determination is based on the following reaction:

ALT/GPT

L-Alanine + 2-Oxoglutarate -------→ Pyruvate +L-Glutamate

Pyruvate 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 L-Alanine 80 mmol/L 2-Oxoglutarate 4 mmol/L

**REAGENT 2 (COLOR REAGENT)** 

2-4-dinitrophenyl hydrazine 1 mmol/L **STANDARD** 

**Pvruvic Standard** 1.2 mmol/L Additional Reagent, but not provided

REAGENT PREPARATION

Sodium hydroxide

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.
- Avoid direct exposure of reagent to light.

## ASSAY

Wavelength 505 nm(490-520 nm) Cuvette 1 cm light path Temperature 37°C

Measurement

Against distilled water

CRESCENT DIAGNOSTICS FACTORY

#### **PROCEDURE**

|   | GPT      |  |  |  |  |  |
|---|----------|--|--|--|--|--|
| Reagent 1 (Substrate)   | 1mL      |  |  |  |  |  |
| Incubate for 5 minutes at 37°C  | <b>L</b> |  |  |  |  |  |
| Serum   | 0.2mL    |  |  |  |  |  |
| Mix and incubate at 37°C for 30 minutes   |          |  |  |  |  |  |
| Reagent 2 (Color)   | 1 mL     |  |  |  |  |  |
| Mix and let 20 minutes at room temperature  |          |  |  |  |  |  |
| NaOH 0.4N   | 10 mL    |  |  |  |  |  |
| Mix, wait 5 minutes. Measure under conditions identical to tho used for the standard curve. The color intensity stable for one hour |          |  |  |  |  |  |

#### CALCULATION

From absorbencies, read unit of GPT 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 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 |     |     |     |     |     |     |  |          |   |    |    |    |     |  |
|  |     |     |     |     |     |     |  | GPT U/mL | 0 | 25 | 50 | 83 | 126 |  |

## LINEARITY

When GPT exceeds 126 U/mL, re-measure diluting the sample 1:10 in 9 g/L Sodium chloride.

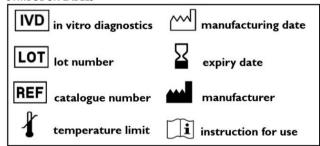
## **NORMAL RANGE**

GPT/ALT: 5-30 U/mL

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

0.4 mol/L