

PRODUCT CODE
CZ004

INTENDED USE

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

METHOD

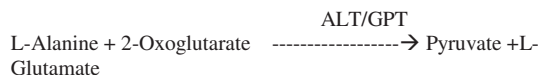
COLORIMETRIC, REITMAN-FRANKEL METHOD

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:



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

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° 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

PROCEDURE

	GPT
Reagent 1 (Substrate)	1mL
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 those 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

QUALITY CONTROL

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

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
	Catalogue Number		Pack Size
	Expiry Date		Volume
	Storage Condition		Lot Number
	Instruction for Use		In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests		For Single Use Only
	EC Representative		European conformity

BIBLIOGRAPHY

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

