Cromatest

LINEAR

GOT color CE GPT color CE

REF 1130005	REF 1132005
2 x 50 mL	2 x 50 mL
CONTENTS	**CONTENTS**
R1a. Reagent 1 x 50 mL R2. Reagent 1 x 50 mL R3. Reagent 2 x 50 mL CAL.Standard 1 x 3 mL	R1b. Reagent 1 x 50 mL R2. Reagent 1 x 50 mL R3. Reagent 2 x 50 mL CAL.Standard 1 x 3 mL
For *in vitro* diagnostic use only	

GOT/GPT color

REITMAN-FRANKEL

Colorimetric method

ENDPOINT

PRINCIPLE

Aspartate aminotransferase (GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxalacetate.

$$\text{L-Aspartate + 2-Oxoglutarate} \xrightarrow[\text{pH 7.4}]{\text{GOT}} \text{L-Glutamate + Oxalacetate}$$

Alanine aminotransferase (GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.

$$\text{L-Alanine + 2-Oxoglutarate} \xrightarrow[\text{pH 7.4}]{\text{GPT}} \text{Glutamate + Pyruvate}$$

The transaminase activity is proportional to the amount of oxalacetate or pyruvate formed over a definite period of time and is measured by the reaction with 2,4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution[1].

REAGENT COMPOSITION

R1a **GOT substrate**. Phosphate buffer 100 mmol/L pH 7.4, L-aspartate 200 mmol/L, ketoglutarate 2 mmol/L.

R1b **GPT substrate**. Phosphate buffer 150 mmol/L pH 7.4, L-alanine 200 mmol/L, ketoglutarate 2 mmol/L.

R2 **DNPH.** 2,4-Dinitrophenylhydrazine 1 mmol/L. Color developer. **C R:34/35**

R3 **4N NaOH** (10x). Sodium hydroxide 4 mol/L. **C R:34/35**

CAL **Pyruvic standard.** 1.8 mmol/L. Secondary standard.

STORAGE AND STABILITY

Store at 2-8ºC.
The Reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

The substrates, standard and color developer are ready-to-use.

Working 0.4 N NaOH solution. By means of a funnel pour the contents of the 10x concentrate 4N NaOH preparation into a 2- liter volumetric flask, rinse the bottle with some volumes of distilled water, complete to the mark and mix. The solution warms up. Let stand till reaches room temperature and complete to the volume. Mix again and store in a well capped polyethylene bottle at room temperature.

SAMPLES

Serum free of hemolysis.
Transaminases are stable in serum 24 hours at room temperature and for 1 week at 2-8ºC.

INTERFERENCES

- Samples with patients under hemodialysis, severe vitamine B deficiency or with related pathologies, lead to an underestimation of GOT and GPT values.
- Highly elevated levels of hemolysis are not suitable for testing.
- Other drugs and substances may affect the GOT and GPT values.[2]

MATERIALS REQUIRED

- Photometer for measurements at 505 nm ± 15 nm.
- Thermostatic water bath set at 37ºC (± 1ºC).
- Stopwatch.
- Pipettes of 5.0 mL, 1.0 mL and 0.1 mL.
- Glass tubes.

PROCEDURE

1. Bring reagents and samples at room temperature.
2. Pipette into labelled tubes:

TUBES	Blank	GOT	GPT
GOT substrate	0.5 mL	0.5 mL	-
GPT substrate	-	-	0.5 mL

Warm to 37ºC into the bath for 5 min.

Add:

Serum	-	100 µL	100 µL

Mix. Return to bath at 37ºC for: **60 min.** **30 min.**

Add:

DNPH	0.5 mL	0.5 mL	0.5 mL

Mix. Stand for 20 min. at room temperature.

Add:

NaOH 0.4 N	5.0 mL	5.0 mL	5.0 mL

Invert to mix. Stand for 5 min. at room temperature.

3. Read the absorbances (A) of the samples against a water blank (Note 1).

The color is stable for at least 1 hour.

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687

CALCULATIONS

From absorbances, read units of GOT or GPT from the corresponding curves.
For activities higher than 200 WU (GOT) or 100 WU (GPT) repeat the test diluting the sample 1:10 with saline and assayed again. Multiply the results by 10 (Note 2).

UNITS

The conversion of colorimetric units into UV units obtained by a kinetic optimized method (IFCC, 1985) cannot take place by the use of a factor as in the classic UV Karmen procedure (1995).[3,4]

REFERENCE VALUES[1]

Serum

GOT/AST	8-40 WU/L
GPT/ALT	5-30 WU/L

Results between 40-50 WU (GOT) or 30-40 WU (GPT) are considered borderline values.

QUALITY CONTROL

To ensure adequate quality control (QC) with this method, the inclusion in each run of controls (normal and abnormal) with assayed values will help to find the equivalence between units.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of GOT/GPT. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of GOT/GPT. Assayed.

CLINICAL SIGNIFICANCE

The group of enzymes called transaminase exists in tissues of many organs. Necrotic activity in these organs causes a release of abnormal quantitaties of enzyme into the blood.

Since heart tissue is rich in glutamic oxalacetic transaminase, myocardial infarction results in high GOT activities in the serum.

The liver is specially rich in GPT. This enzyme measurement is used primarily as a test for hepatitis. With infectious hepatitis the GPT activity in serum is greater than of GOT, but both activities usually are increased. Thus, generally speacking, the GOT value is used for diagnosis of myocardial infarction, while the GPT value is useful in diagnosing infectious hepatitis. Neither test is specific.

NOTES

1. The tests may be read against water set at zero absorbance. However, the technic of running a reagent blank and setting this at 0.250 A helps to compensate for small changes that might occur in the reagents or instrument.
2. Dilutions of serum do not always give values in exact proportion to the magnitude of the dilution.
3. This standard, when used as described gives a curve which agrees more closely with the reference method than does the 2 mmol/L original standard recommended by Reitman and Frankel.

REFERENCES

1. Reitman, S. and Frankel, S. J. Clin. Pathol, 28:56 (1957).
2. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).
3. Bergmeyer, H.U., HØrder, M., Rej, R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 2. IFCC Method for Aspartate Aminotransferase, J. Clin. Chem. Clin. Biochem. 24, 497-510.
4. Karmen, A. J. Clin. Invest. 34 : 131 (1955).

Calibration setting

1. Pipette (mL) into labelled tubes:

TUBES	PYRUVIC STANDARD	GOT SUBSTRATE	H_2O	WU/mL GOT	WU/mL GPT
1	-	1.0	0.2	0	0
2	0.1	0.9	0.2	24	28
3	0.2	0.8	0.2	61	57
4	0.3	0.7	0.2	114	97
5	0.4	0.6	0.2	190	-

2. Add to each tube 1.0 mL of DNPH. Mix. Stand for 20 minutes at room temperature.
3. Add to each tube 10.0 mL of 0.4N NaOH. Mix. Stand for at least 15 minutes.
4. Blank the instrument with distilled water.
5. Using linear graph paper plot the *units of activity* shown above against their respective *absorbances (A)* at 505 nm. Check the curve periodically (Note 3).

B1130-1/0412
R1.ing

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687